



UNITED STATES AIR FORCE  
ARMSTRONG LABORATORY

LAUNCH AREA TOXIC RISK ANALYSIS  
PROGRAM (LATRA)  
TOXICOLOGY REVIEW

Joseph K. Prince  
Teresa R. Sterner  
Erik K. Vermulen

OPERATIONAL TECHNOLOGIES CORPORATION  
1010 WOODMAN DRIVE, SUITE 160  
DAYTON OH 45432

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Occupational and Environmental Health  
Directorate  
Toxicology Division  
2856 G Street  
Wright-Patterson AFB OH 45433-7400

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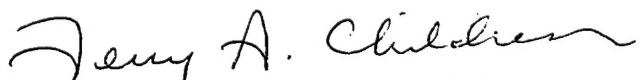
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## EXECUTIVE SUMMARY

The toxic cloud corridors, generated during normal and catastrophic rocket launch scenarios and the possible impact on human health is a source of concern to the US Air Force. Therefore, a literature search of three species of rocket emissions was performed to identify health effects information useful to HQ Space Command for managing the risk from these toxic clouds. The literature retrieved on the three compounds of interest, hydrogen chloride (HCl), nitrogen oxides (NO<sub>x</sub>) and nitric acid (HNO<sub>3</sub>), was evaluated to identify the toxic responses associated with inhalations from such exposures. Animal and human data generated in toxicity studies were assessed, indicating that the respiratory tract tissue was the target of exposure to these compounds. Based upon the available toxicity information, acceptable levels of exposure were proposed. The toxicological information database under review and proposed recommendations were presented for consideration to the National Research Council-National Academy of Science Committee on Toxicology.

In addition to the biological response profile, the uncertainty of the exposure level and duration impacts the public health risk estimate. Our analysis of the exposure response function (ERFs) of the LATRA model addressed response uncertainty only. This function reflects the responses from absorbed dose but does not address the possible responses to variations in dose rate. That requires additional toxicological information, such as defining an ERF value according to changes in exposure duration. Also, the use of different ERFs for various receptors is not supported by the empirical toxicological database.

Recent publications using a logistic regression model to examine the short term toxicity of HCl exposure were reviewed to determine the possible utility of this regression model for understanding the risks of exposures to various rocket emissions (see Fig. 1-1). The short term logistic regression model (STLRM) used was studied and considered useful because it considered exposure concentration and duration and described different levels of severity of adverse physiological effects among exposed test subjects. Accordingly, an analysis of human and animal NO<sub>2</sub> toxicity data cited in the references was performed using the STLRM approach (see Section 7).

The exposed human groups cited in the studies appeared to be typical of, but not ideally, that of an expected receptor population. Additionally, reported health effects among humans and animals indicate  $\text{NO}_2$  impacts the same target organ tissue, the respiratory tract. Further analysis of the physiological response among  $\text{NO}_2$  exposed humans indicates no readily discernible difference in physiological response between normal and supposedly sensitive human subjects.

Adoption of a multiple tier level of response classification allows specific recognition of possibly reversible but statistically and biologically significant responses to be identified without assuming responses are severe adverse responses as opposed to a normal adaptive defense mechanism. The multiple tier classification of severity of response allows this type of regression analysis to be performed with a minimum amount of subjective judgments. Adoption of the STLRM model approach allows HQ Space Command to conduct risk management program activities in a manner compatible with recent EPA practices. The analysis suggested the model can be useful for describing the exposure response function, but for full utility requires a satisfactory database and minor modifications to the classes of severity and the probability equations.

The existing data was assessed and presented as a suggestive relationship of most likely estimate curves for HCl and  $\text{NO}_2$  (Fig. ES-1 and ES-2) . An additional effort with a larger database may be required to confirm the proposed methods for these or other rocket toxicant emissions. Review of the available literature indicates an adequate body of data does not exist for  $\text{HNO}_3$  to provide useful dose response curves. Likewise, adverse effects from exposures to multiple species is extremely limited and an analysis of the cellular responses for the three chemicals does not support any method for combining exposures other than the normal additive assumption. Estimation of the expected variation of exposure concentrations and peak concentration duration is needed if effective risk management under the new EPA Clean Air Act Risk Management Plans can occur.

Based on these exposure levels further development of ERFs for HCl and  $\text{NO}_2$  are possible for the LATRA Model. The toxicology database for  $\text{HNO}_3$  was insufficient to prepare either a useful toxicological or logistic regression model analysis. A recommended level of exposure is not proposed for  $\text{HNO}_3$ , it should be treated as  $\text{NO}_2$ .

The resulting analysis of toxicity data, using human and animal toxicity data, cited references and the STLRM analytical procedure indicates the following to be acceptable levels for an exposure of one hour or less.

**Table ES-1**  
**RERA TOXICOLOGICAL DATA ANALYSIS**

<b>SPECIES OF CONCERN</b>	<b>ON-SET OF RESPONSE(PPM)</b>	<b>MAXIMUM EXPOSURE (PPM)</b>
HCl <sup>B</sup>	4.1	166 <sup>A</sup>
NO <sub>x</sub> <sup>C</sup>	0.6	36
HNO <sub>3</sub>	N/A	N/A

A = 95 % confidence level that 95% of population will not exceed moderate response from a one (1) hour or less exposure.

B = Human equivalent concentration, Table 9, USEPA, 1991.

C = Animal mortality data

Information gaps have been identified and recommendations for additional research were provided for HCl, NO<sub>2</sub> and HNO<sub>3</sub>. Additional empirical research is required on animal and human response to short term exposures at moderate dose levels. A well developed methodology integrating exposure dose with varying duration of time for short term acute exposures for other emissions of concern should be explored and clarified for use in the launch scenario. The additional data will be required to provide an adequate measure of support to proposed levels of acceptable exposures from the rocket toxicant emissions.

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## 1.0 INTRODUCTION

### 1.1 BACKGROUND

Range safety has been a public policy issue from the very beginning of the space program. Public Law 81-60 and its Legislative History dictated that the public risk from space operations should be no greater than from aeronautical operations (EWR 127-1). EWR 127-1, Range Safety Requirements further define a risk management criteria as thirty causalities in a million ( $30 \times 10^{-6}$ ) as being the acceptable launch risk without high management review. This is an implicit recognition of the dynamic balance the range operators must manage between public safety and national security. As our space launch ranges have been impacted by encroachment and the size of the launch vehicles has increased, the potential for public health and safety impacts has grown. There are two general scenarios which drive potential impacts: (1.) cold spills of volatile liquid or gaseous propellants or (2.) combustion products from the rocket motors or engines. There are numerous studies of the by-products of normal combustion; JANNAF indicates approximately 5% of the gaseous products from rocket motors was hydrogen chloride (HCl) and 0.2% oxides of nitrogen ( $NO_x$ ). Risk management scenarios must be assessed where the vehicle weight varies from 2,000 to 4,500,000 pounds. HCl emissions for the Space Shuttle have been estimated at 35,000 kg or 77,000 pounds per launch. Measurements within the cloud during a normal Titan III launch in 1978 revealed the cloud stabilized at 1.22 km or 0.76 miles within 950 seconds with peak HCl concentrations varying between 2.5 and 16 ppm (Sebacher et al., 1979). They reported early partitioning of the HCl into aerosols which plated out, however residual HCl remained as a gaseous form at a relative humidity of less than 70%. It is believed that much higher HCl concentrations have been predicted to occur. Catastrophic failure of the Titan missiles can release significant quantities of nitrogen tetraoxide and Aerozine 50.

Managers within the US Air Force, the Space Command and the range commanders at the Western and Eastern Range are concerned over any impact to the health of residents surrounding the launch ranges. To protect public health, both, the Eastern and Western range launch commanders currently depend in part on two probabilistic models for assisting in their decision making. The Launch Area Toxic Risk Analysis [LATRA] model, and the

Rocket Effluent Exhaust Diffusion Model [REEDM]), provide estimates of possible human risk from these emissions. Regulatory oversight to the risk management practices has been informal. However, with the passage of the Risk Management Planning (RMP) under the Clean Air Act (40 CFR 68, sect 112 [r], 20 April 96), compliance requirements have been implemented. A National Advisory Commission is developing recommendations for Acute Exposure Guidelines (AEGLs) to be incorporated in the Act (BNA, 1996). During their August 1996 meeting, they reportedly developed draft guidelines for hydrazine now in internal review. This evolution in regulatory status is expected to influence the relationship the launch operators have with the surrounding public. The RMP contains specific methodologies for the determination of risk and require release of the information to the general public. Prior levels of "tolerable risk" generated in response to the Legislative History may be irrelevant during future risk communication. Issues such as perceived risk, risk - benefit, trust, catastrophic potential, fairness and degree of control will have a significant influence on the acceptable risk levels (Sandman, 1993) .

LATRA provides probabilistic estimates of mishaps and health effects responses. In one function, LATRA models the toxic releases for nominal launch and probable releases for catastrophic events. It also incorporates dispersion data developed from another model (REEDM) and toxicological information to prepare risk profiles and expected number of casualties.

REEDM predicts airborne movements of the emission products and exposure levels. It projects a proposed toxic corridor from historical and immediate meteorological data. The gaussian REEDM dispersion model acts as a sub-routine of LATRA. It uses a covariant matrix database of historical monthly weather conditions and flow type. It incorporates recent weather conditions (4.5 hours prelaunch), and provides estimates of the probable movement of a chemical plume, based on distributions of the current and previously developed information (Philipson, verbal communication). This output suggests toxic corridors of the potential distribution of exhaust products by concentration and time across exposed receptors.

A major parameter in the LATRA model is the exposure response function (ERF). The ERF describes the probability of occurrence of a certain level of severity for an effect corresponding within a population model categorized by sensitivity levels, as a logarithmic function of exposure. The LATRA output is "expected casualties" and risk profiles for each susceptibility level and each potential level of severity. These models provide risk estimate information to assist launch commanders in deciding whether or not to issue the launch ("Go/No Go") command. However, reservations persist on the approach and data used in developing the model. Accordingly, an *ad hoc* Rocket Exhaust Working Group and an NAS-COT/Sub-committee on Rocket Emissions have convened to address the underlying toxic response data. They have recognized several issues:

- Neither the model's validity nor its' sensitivity have been established.
- The ERF curve model needs to be examined for accuracy and goodness of fit with well defined high quality dose response data; it should be determined if the model fits all toxics of interest.
- Exposures variations may be treated as a time weighted average (TWA) or peak ceiling concentration.
- Duration and/or levels of exposures are imprecise or unknown.
- Definition of mild, serious or significant toxic effects, and of normal and sensitive receptors are lacking.
- "Expected casualties" implies fatalities, selection of other responses is needed for emergency planning.

## 1.2 OBJECTIVES

Several issues and objectives have been proposed for the total Rocket Exhaust Risk Assessment (RERA) effort. The sponsor of this effort asked for review of the role of the ERFs, assessment of the specifications of the ERF, review of the literature on acute toxicology of the gases (HCl, the Oxides of Nitrogen [NO<sub>x</sub>], and Nitric Acid [HNO<sub>3</sub>],) individually and jointly, recommendations for enhancements to the ERF and documentation of research needs. Accordingly, the first task is to identify toxic or pathological health effects that may occur among humans from such exposures; especially to identify the acute

effects that may accompany short term/intermittent exposure levels. Multiple simultaneous exposures may elicit additive and/or synergistic effects. Responses may be more significant or occur at lower levels of exposure for sensitive populations. Acid gases, particularly nitrogen dioxide ( $\text{NO}_2$ ) occur in the ambient environment (Lindvall, 1985). Homes with gas cooking may have between 0.25 and 1.0 ppm  $\text{NO}_2$  for periods up to two hours daily. Ambient atmospheres on the west coast (Los Angles basin) may have  $\text{NO}_2$  levels at 0.8 ppm for one hour occurring in conjunction with ozone ( $\text{O}_3$ ), a known irritant. These "natural" levels are near the threshold for responses in humans and provide a baseline for our risk assessments.

The following are some specific objectives for this effort:

- a. Identify the human equivalent concentration responses for several animal species at the frank effect level for acute exposures over short periods on each of the gases. Identify any joint exposure responses. Assess concentration kinetics for dose averaging.
- b. Identify the human equivalent exposure level of no significant response for short duration.
- c. Identify the human equivalent exposure for the on-set of reversible but significant biological responses for short duration responses. Differentiate between response levels.
- d. Identify comparable response levels for sensitive populations (aged, young, chronic respiratory diseased) and their relationship to normal population responses.
- e. Determine the appropriateness of the mathematical form of the ERF for short duration acute exposures of acid gases.
- f. Identify the thresholds for significant biological responses from biochemical and physiological endpoints.
- g. Assess sensitivity of exposure variations to expected responses.
- h. Determine the information gaps and recommended research needs.

The toxic and pathological health effects information of airborne exposures to the gases/aerosols described below are developed in three separate sections. Section 3 focuses on HCl, Section 4 focuses on NOx, while Section 5 will focus on  $\text{HNO}_3$ . The Sections are further organized into several subsections. The toxic or pathological effects to

the respiratory system of humans and animals is described according to tissue affected; Nasopharyngeal-Sinus or Bronchiolar-Alveolar. Pulmonary effects will be discussed by the level of exposure as related to the effect elicited by organ, then by tissue, by cell and by biochemical activity. Toxicopathology will be classified as immediate or latent. Effects will be further defined as a nuisance irritation, a mild effect and/or a significant effect; and effects will be further characterized as transient, reversible or irreversible.

Exposures to the acid gases are expected to be a rare event (once in a lifetime) and use of current planning guidelines may be misleading. The basis of the current guidelines will be documented. Each of the organizations use their own combination of factors to deal with variations in exposure levels over time, variations in biological sensitivity and variation in acceptable response levels. One of the critical concerns for the launch scenario is the time varying levels of exposure during a single event. In most acute exposures chemicals do not behave according to Haber's Law ( $R=CxT$ ) but they do seem to behave better when the concentration is adjusted for potency to  $R=C^n \times T$  where  $n$  may vary from  $n < 1$  to  $n > 3.5$  (Alexeef, 1992). In the occupational scenario, the criteria values incorporate a ceiling value to deal with these excursions. In the emergency planning area, the basis for the ceiling values may not hold for very short duration exposures. The EPA looks at lifetimes but the extended duration of exposure will play a greater role. However, the recent approach used by the EPA in modeling dose - response - duration curves may be more appropriate for application to these exposures. The behavior of these gases at the lethal levels will be assessed under short duration exposures. Likewise the behaviors at the threshold of reversible biological effects will be assessed.

The protocol used to search the electronic literature databases is in Attachment 1. A listing of all references identified may be obtained from the authors. Those references cited in the report are listed in Section 7.

### **1.3 TREATMENT OF TIME VARYING EXPOSURES**

One of the principal tasks required for effective analysis is an assessment of the functional form of the ERF as it relates to acute toxicology in public emergency response

planning. Currently, the ERFs are generated for a response using a log-probability plot (Philipson, 1996). It is assumed the functional relation is a straight line defined by two end points. The upper point is taken where 99% of the population is believed to respond; the lower point is a level where 1% of the population will respond. In the past these were assumed to be the Immediately Dangerous to Life and Health (IDLH) level and the short-term Public Emergency Guidance Level (SPEGL) for the upper and lower points, respectively. During the last year, other values were assessed to better describe the recent toxicological findings (Hinz, 1995).

Numerous authors have identified the log function as appropriate to the short-term exposure scenario (Alexeef, 1992, 1993, US EPA, 1991). Response rates for non-cancer endpoints are often poorly reported so that multivariate dose response models are seldom generated. Accordingly, authors have focused on the severity of effects when describing dose-response analysis, describing these responses, qualitatively. Given the line of sight description, many others felt this method was somewhat arbitrary and categorical regression was introduced to remove these objections. Hertzberg (1989) suggests there is sufficient information to support the logarithmic plot and proposed the form of regression used in risk estimation be the logistic model. The following model is assumed:

$$P(s>i) = 1/(1+\exp[-a-X^b]), \text{ where}$$

$s$  = severity of response,

$i$  = severity category,

$L$  = number of severity levels,

$X$  = independent variable such as log dose

$\beta$  = parameter estimate of independent variable (common slope parameter)

$\alpha_i$  = parameter associated with response category  $i$ .

Therefore the logistic dose model will be  $L$  equations with a common slope parameter  $\beta$ .

As currently used in the LATRA model, probability of response is assumed to be constant with dose regardless of exposure duration. It seems to assume the dose absorbed equates to a response. Concentration in air is substituted for dose, assuming the dose rate has no influence. The toxicology literature reports response levels at a variety of doses in

which Haber's Law may not hold true. Over the last two years, concern was expressed for the sensitivity of ERFs to very short-term peak exposures (with integrated dose) and whether ceiling values documented in TLV criteria are relevant. Given the effects of exposure duration, Haber's Law needs to be modified to  $R = C^n \times T$ , where  $n$  (a function of the material) varies from 0.8 to 3.5. The EPA proposed use of one of two models (Weibull, log-logistic) in generation of their dose-duration-response models (US EPA, 1991). Either model will simplify to the logarithmic relationship used by LATRA when duration is not a consideration. The EPA approach recognizes the time-concentration sensitivity issue and permits use of actual empirical information without the cascading effects of safety factors involved in adopting TLV-like methodologies. In setting their relationship function, EPA used animal and human data but corrected all exposures to Human Equivalent Concentrations (HEC). The factors used in the correction depend upon the mechanism and site of toxic effect. With the acid gases some researchers have noted both lung and non-lung responses. Others have noted the non-lung responses could be related solely to the respiratory dysfunction. Assuming a respiratory only response at moderate to low doses seems appropriate for determining the HEC ratios. The EPA's model for HCl is duplicated in figure 1.1. Similar empirical toxicology information was extracted from the literature to generate a curve for NOx at figure 1.2. Supporting data for figure 1.2 is contained in Attachment A.

Integration of time varying dose presents another problem to assessing population risks. Although we have no information on how concentrations are expected to vary over typical scenarios at a particular location, integrating the dose absorbed to predict response will be critical. Both the time weighted Threshold Limit Value/Short-Term Exposure Limit (TLV/STEL) and the SPEGL methodology have attempted to deal with assessing the response from highly varying exposure concentrations. Exposures of less than 5-15 minutes have not been studied due to the difficulty of, controlling highly transient exposure conditions and measuring responses accurately, during bioassay studies. This has been compounded by the lack of precise analytical techniques in the older literature. Physiologically, exposures for less than a minute seem to lack meaning. Breathing may have been voluntarily interrupted or patterns altered to yield no valid information on the

absorbed dose. Concern has been expressed that predictions from launch operations yield highly variable concentrations, yet for the acid gases 15 minute exposures at high concentrations can incapacitate or cause significant response even when a one hour time weighted average suggests little risk (Alexeeff, 1992).

Studies with NO<sub>x</sub> have shown non-linear behavior between response and low concentration exposures (Bylin, 1995). Lethality, in another study, was observed to be higher with short exposures to high concentrations but underlying structural damage was higher with longer duration to moderate levels (Hine, 1970). Therefore, the time varying response at different levels of significance of the endpoints can be considered to be independent.

#### **1.4 TREATMENT OF SENSITIVE POPULATIONS**

The current direction used in the LATRA model is to generate separate ERFs for each target population. Healthy adult workers may be one population of concern where the aged, youth, asthmatics, and etc. may be other populations of concern. Differentiation between sensitive populations is a function of the material properties and some endpoint. Those materials with the potential to cause mutagenic responses, for example, would lead one to assess the reproducing female population as potentially sensitive. Likewise, acid gas exposures would suggest that those with cardiopulmonary disease can be considered as potentially sensitive. Although the data in the literature is incomplete, there is some information on the responses of humans to the materials in question. No effects levels have been determined for both healthy and sensitive populations in the literature, but have been equivocal.

Biological variability within any artificially defined sensitive sub-population confuses the response observed from any particular dose. Sensitivity varies within any sensitive population from mild to severe. Development of ERFs to reflect probability of response within a sensitive group would require significant toxicology data to identify on-set of reversible and irreversible responses over dose and duration. Also, a single compound may have more than one endpoint defining sensitive populations. The principal issue with

populations is a concern that the on-set of response may occur at lower doses than with "normal" populations. Uncertainty factors have been routinely used in criteria development to protect the entire population. Values of 3x or 10x have been used by EPA when information is sparse in supporting their extrapolations. An alternative approach (Alexeeff, 1993) is to use available empirical information to calculate adjustment factors to modify the practical thresholds for these concerns. In this way the cascading of uncertainty is avoided and the criteria is set for sensitive populations based on measured comparisons. Clearly, it would be desirable to have the full response characterization of the various sensitive populations, but in its absence using the ratio of on-set of response may provide better insight than application of policy driven uncertainty factors. The proposed model is  $CL_s = CL_n / RSF$  where the RSF (Response Severity Function) is the ratio of the on-set of endpoint at the representative concentration and duration of exposure between sensitive ( $CL_s$ ) and normal ( $CL_n$ ) populations. Selection of the RSF would be material and scenario specific. This proposed model may overly simplify the complexity of sensitive populations but in the absence of good empirical information it does derive its output based on the best information available. Since the objective of the ERFs is to provide guidance on the potential catastrophic response of the public, systematic exclusion of part of the population in the initial recommendations seems unwarranted. If as part of response actions the decision maker opts to exclude part of the population as absent or not at risk, then the RSF could be used for assessment of the subpopulation only. Table 1.1 presents RSF values taken from the literature for the compounds of interest.

These estimates are not rigorous but only suggestive. Ratios for the on-set of response at low concentrations provides information on setting the boundaries for the exterior envelope where no effects are predicted but tells one nothing about the relative sensitivities of parts of the population to higher exposures. It is possible that the sensitive part of the population could have more severe responses to intermediate concentrations than the remainder of the population.

**Table 1.1 Response Severity Factors for Sensitive Populations**

Compound	RSF Value	No. of Studies	Comments
HCl	1.0	1 (Stevens, 1992)	10 Subj. @ 45 min to 0.8, 1.8 ppm
NOx	2.0	6 sensitive, 6 normal	most frequent normal 0.6 ppm
HNO <sub>3</sub>	unknown		

However, biological variability may mask the differences. Case studies have shown the induction of asthma like symptoms in normal adults at relatively high concentrations on a single exposure (Boulet, 1988). For HCl the RSF was suggested to be 1.0 based on the single study with asthmatics. The on-set of response in the total population is suggested to be between 0.2 and 10 ppm with 5 ppm as the likely odor threshold. A threshold of 0.4 ppm for ocular effects was estimated (Stevens, 1992). It has been estimated from human data that work can continue in concentrations of 10 to 50 ppm HCl for 1 hour but with difficulty (US EPA, 1991).

### **1.5 DIFFERENTIATING SEVERITY OF RESPONSE**

During generation of the ERFs it became apparent that the endpoints of the various expected injuries were not always equivalent. The original approach with the ERFs was to define responses with comparisons between the accident caused injuries from blasts or fire to equivalent toxicity endpoints. One underlying feature which impacts this goal was the unexpected nature of blast injuries and the knowledge that toxic endpoints could be experienced with a "normal" launch. Both normal and catastrophic events have been common features in dealing with emergency planning. Public perception and acceptance becomes an element in determining the appropriate endpoints.

The original objective to differentiate between mild and significant endpoints may have become irrelevant. Recently, the regulatory agencies have adopted language for emergency planning (EPA, 1996) which discriminates between one set of endpoints as detected, reversible or non-disabling and those which are disabling, irreversible injuries. Injuries were defined as a requirement for treatment beyond first aid or hospitalization. The disabling or irreversible toxic endpoints were found to be equivalent to accidents generating

one pound per square inch (psi) over pressure or 5 kilowatts per square meter (kW/m<sup>2</sup>) thermal burden.

Other authors have defined four levels of response starting at the no effect level, the biologically significant but reversible level, the biologically significant non-reversible level and death / damage to underlying structure (US EPA, 1991). The complexity of biological response to a stressor yields a multiple of observable endpoints. Normally, empirical information can be assessed to fall into one of these categories and the boundaries defined statistically. Each of the responses can be assessed to determine how they vary with concentration and duration of exposure. The table 1.2 presents the classifications. The break between Class 2 and Class 3 seems to equate to the endpoints referenced in the second paragraph of this section.

**Table 1.2 Classification of Response**

Class	Level	Definition
1	No Effect	No or Reversible Effects
2	Minor	Biologically Significant, Reversible
3	Moderate	Biologically Significant, Non-reversible
4	Severe	Death, Damage to Structure

## **1.6 COMPARISON OF STANDARDS/CRITERIA FOR HCl, NO<sub>2</sub> AND HNO<sub>3</sub>**

### **The National Institute for Occupational Safety and Health (NIOSH)**

**Recommended Exposure Levels (REL)/Immediately Dangerous to Life and Health (IDLH):** NIOSH RELs are based on NIOSH evaluations of all relevant scientific information about a given hazard and are conveyed to the Occupational Safety and Health Administration (OSHA) for use in promulgating legal standards. The Documentation for IDLHs, NIOSH, 1994 notes that the original HCl IDLH of 100 ppm was revised to 50 ppm based on acute inhalation toxicity data in humans (Flury and Zemik, 1931; Henderson and Haggard, 1943; Tab Biol Per 1933). The nitrogen dioxide original IDLH of 50 ppm was revised to 20 ppm based on the same acute toxicity data cited in the original IDLH, which was Patty 1963. The revised IDLH for NO<sub>2</sub> is noted as a conservative value due to the lack of relevant acute toxicity data for workers exposed above 20 ppm concentrations. The nitric

acid IDLH was revised to 25 ppm based on acute toxicity data in human as reported by Gekken, 1980 (430 mg/kg oral lethal dose is equivalent to an inhalation dose of 2,300 ppm for 30 minutes) and no adverse effect for animals at 24 ppm (Diggle and Gage, 1954). This revised IDLH for  $\text{HNO}_3$  may be conservative due to the lack of relevant acute inhalation toxicity data for workers.

Sensitive populations are not considered for the IDLH. The IDLH is only for the purpose of respirator selection and represents a maximum concentration from which, in the event of respirator failure, one could escape within 30 minutes without experiencing any escape-impairing or irreversible health effects. The IDLH values were developed for the occupationally exposed and not the public. These workers know the hazards they are working with and have the necessary respiratory equipment, procedures and training to use them if there is an accidental release. Conversely, the public would not be aware of the potential hazard, not have the respiratory equipment nor be pre-selected for wear of respiratory protection.

Ideally, human acute exposure data would be the most appropriate, however, animal studies generally provide the best data for estimating the potential human response to a given exposure. The documentation for the  $\text{NO}_2$  and  $\text{HNO}_3$  notes that the original and revised IDLHs were based on limited acute human health evaluations and should be considered conservative values.

**The American Conference of Governmental Industrial Hygienists (ACGIH)**  
**Threshold Limit Value (TLV):** The TLV refers to the airborne concentrations of the substance and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects. However, sensitive populations are not considered in the TLV. Per the 1995-1996 TLV booklet, pg. 2, "a small percentage of workers may experience discomfort from some substances at or below the TLV; a smaller percentage may be affected more seriously by aggravation of a pre-existing condition or by development of an occupational illness....Individuals may also be hypersusceptible or otherwise unusually responsive to some industrial chemicals because

of genetic factors, age, personal habits (smoking, alcohol, or other drugs), medication, or previous exposures. Such workers may not be adequately protected from adverse health effects from certain chemicals at concentrations at or below the TLVs."

Besides the TLV-time weighted average (TWA), which is established for an eight hour workday, the TLV-Short Term Exposure Limit (TLV-STEL) was established as the concentration to which workers can be exposed continuously for a short period of time without suffering from: 1) irritation, 2) chronic or irreversible tissue damage, or 3) narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce work efficiency. *The STEL supplements the TWA limit where there are recognized acute effects and are recommended only when toxic effects have been reported from high short-term exposures either in humans or animals.* The STEL is for a 15 minutes period. A worker can be exposed up to the STEL on a daily basis.

TLV-Ceiling (C) is the concentration that should not be exceeded during any part of the working exposure. If instantaneous monitoring is not feasible, the TLV-C can be assessed by sampling over a 15 minutes period except for those substances that may cause immediate irritation when exposures are short. For irritant gases, only one category, the TLV-Ceiling may be relevant. For other substances, one or two categories may be relevant, depending upon their physiologic action.

TLVs are established based on available information from industrial experience, experimental human studies and animal studies. The basis of establishing the TLV may vary from substance to substance. For example, one compound may protect against reasonable freedom from irritation, narcosis, nuisance or other form of stress. The latest TLV documentation will provide the basis for each TLV and how it was established.

The HCl TLV-C is based on minimizing potential toxicity and the acute irritation (TLV Doc., 1991, Pg. 773). The NO<sub>2</sub> TLV is based on reducing the potential for immediate injury of adverse physiologic effects from prolonged daily exposure to NO<sub>2</sub> (TLV Doc., 1991 pg. 1109). Per the COT NO<sub>2</sub> report, they noted that the TLV (1984) for NO<sub>2</sub> is based on animal

data obtained in 1952 and 1965. The 1991 TLV Doc. cited the same two animal studies. The HNO<sub>3</sub> TLV is based on prevention of ocular and upper respiratory tract irritation and should also preclude dental corrosion (TLV Doc., 1991, pg. 1089).

**American Industrial Hygiene Association (AIHA) Emergency Response Planning Guidance (ERPG):** Only HCl has an AIHA ERPG and its as follows:

**HCl ERPG-1 - 3 ppm, ERPG-2 - 20 ppm, and ERPG-3 - 100 ppm.**

Per the preface to the ERPG, there are hypersensitive individuals who will show adverse responses at exposure concentrations far below levels where most individuals normally would respond. The ERPGs do not contain safety factors that are common in other exposure guidelines such as one to address the variability of human response. The most pertinent information for the ERPG comes from human data where available.

ERPG-1 is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed up to 1 hour without experiencing other than mild transient adverse health effects or perceive a clearly definite, objectionable odor. The rationale for the HCl ERPG-1 was based on available odor threshold data and the National Research Council (NRC) Committee on Toxicology (COT) guidelines (see next section) that indicated HCl concentrations above 3 ppm would cause transient eye and respiratory tract irritation (AIHA, 1994).

ERPG-2 is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed up to 1 hour without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action. The HCl ERPG-2 documentation (AIHA, 1994) noted that HCl levels above 20 ppm but less than 100 ppm would be expected to cause serious eye and respiratory tract irritation.

ERPG-3 is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hr without experiencing or developing life-

threatening health effects. The HCl 100 ppm ERPG-3 was based on animal acute data indicating 100 ppm for one hour may cause pulmonary edema and possible death in the heterogeneous human population (AIHA, 1994).

**NRC COT Emergency Exposure Guidance Level (EEGL) and Short-Term Public Emergency Guidance Level (SPEGL):** The NRC COT has recommended EEGLs and SPEGLs on behalf of the military for unique compounds peculiar to military operations. These guidelines are intended for military personnel operating under emergency conditions. The EEGL is the concentration of the substance in air that may be judged acceptable by DoD for performance of specific tasks during **rare** emergency conditions lasting from 1-24 hours. Exposures at the EEGL might produce reversible effects that do not impair judgment and do not interfere with proper response to the emergency (COT, 1986, pg. 2). The EEGL differs from the ACGIH or OSHA STEL in that the STELs are generally 15-minute limits to which workers may be exposed daily for many years. The EEGL estimation will be based on review of all end points including reproductive in both sexes, developmental, carcinogenic, neurotoxic, respiratory, and other organ-related effects are assessed and the most important one is selected. In the absence of better information, a safety factor of 10 is suggested for EEGLs if only animal data are available and extrapolation from animals to human is necessary for acute, short-term effects or if the likely route of human exposure differs from that of a relevant experiment.

The SPEGL is defined as a suitable concentration for unpredicted, single, short-term emergency exposure of the general public. The COT will take into account the wide susceptibility of the general public including sensitive groups such as children, aged, and persons with debilitating disease. Effects on fetus exposure as well as reproductive capacity of both men and women are also considered. The SPEGLs are generally set at 0.1 to 0.5 times the EEGL. A safety factor of 2 is appropriate for more sensitive groups such as children and the elderly; for fetuses or newborns, a safety factor of 10 is appropriate.

HCl is a strong irritant to humans and because of its solubility in water, acute inhalation exposure are usually limited to the upper passages and are severe enough to encourage voluntary withdrawal from the contaminated atmosphere. At higher concentrations, the bronchioles and alveoli might be penetrated. Prolonged human exposure at 10 ppm was without adverse effect but short term exposure at 35 ppm caused irritation of the throat. Acute animal studies in rat and mouse indicate identical toxic signs, which included irritation to the eyes, mucous membranes and exposed area of skin. Gross examination of the animals post exposure showed emphysema, atelectasis and pulmonary edema. A similar animal study showed pulmonary congestion and intestinal hemorrhage in both rats and mice and thymic hemorrhage in rats. A study with the addition of alumina dust to concurrent exposure to HCl and hydrogen fluoride to simulate exposure to rocket exhaust had no effect on the rat or mouse mortality.

The COT Vol. 7 report on HCl (pg. 24) noted that for quantitative predictions, Alarie suggested that a concentration capable of evoking a 50% decrease in respiratory rate (RD50) in mice could induce intolerable sensory irritation and incapacitate humans; that at 0.1 RD50, humans would report slight stinging or burning of the eye, nose and throat, but that this exposure would be tolerable, and that at 0.01 RD50, very slight or no sensory irritation would occur. However, Potts and Ledereer (1978) reported that the RD50 concentration in mice was not incapacitating to humans. The RD50 for mice is 309 ppm.

In the Committee recommendations section of the report (pg. 27), COT cited that Alarie and others recommend a short-term exposure limit for irritant gases be based on one-tenth of the mouse RD50, and for HCl this would be 31 ppm. The COT then recommended a 1-h EEGL of 20 ppm because of the paucity of human and animal data. They proposed a 24-h EEGL of 20 ppm, because the toxicity of HCl at low concentrations such as 20 ppm did not follow Haber's Law. COT recommended a 1 ppm SPEGL for both 1 h and 24 h to avoid adverse effects to sensitive populations (infants, children, elderly, people with bronchitis or upper respiratory infection). Exposure in utero would not occur at the 1 ppm level because of maternal protection.

For NO<sub>2</sub>, COT (1985), a recommended SPEGL of 1hr at 1 ppm based on a summary of the human exposure data that would not pose an important increase in airway resistance in an emergency.

**U.S. Environmental Protection Agency (EPA):** The US EPA, Federal Emergency Management Agency (FEMA), US Department of Transportation (DOT) Technical Guidance for Hazardous Analysis: Emergency Planning for Extremely Hazardous Substances (1987) suggested public exposure guidance levels or levels of concerns (LOCs) could be based on one-tenth of the IDLH. Other possible LOCs could be estimated from ACGIH TLVs, guidelines developed by the National Research Council of the National Academy of Sciences and the AIHA Emergency Response Planning Guidelines (ERPGs). The US EPA, FEMA, US DOT technical guidance recognized that the IDLH were not designed to protect the general population and did not account for more sensitive individuals (young, aged and infirm). The appendix D also noted that the COT EEGLs are established with safety factors to reflect the nature and quality of data and account for animal to human extrapolation (SF = 10), and for other routes of exposure such as oral data vs. human inhalation exposure when it is the most likely. The EPA uses the ERPG-2 or the LOCs based on the 1987 IDLH (the 1994 IDLH methodology was not reviewed by the EPA's Scientific Advisory Board for emergency public planning).

The U.S. EPA will adopt Acute Exposure Guideline Limits (AEGLs) for risk management plans required by the Clean Air Act Amendment. These AEGLs are being formulated by a National Advisory Committee. This 30 member federal advisory committee first met June 19, 1996 and will generate AEGLs for 400 chemicals over the next 5 to 8 years for use by federal, state, and local governments and others in responding to accidental chemical release. The AEGLs will be for four time periods ranging from 30 minutes to eight hours with three exposure levels:

AEGL-1 - level at which a substance can be detected and exposure will cause reversible, non-disabling effects.

AEGL-2 - the level at which disabling and irreversible effects occur.

AEGL-3 - levels at which death is expected.

The committee has developed interim exposure levels for fluorine and ammonia and have considered hydrazine. All interim exposure levels will be reviewed by the National Research Council's standing COT and published in the Federal Register for public comment prior to publication as final exposure levels (BNA 1996). According to Dr. Gephart, a AGEL committee member, the committee does not currently have military representation.

Table 1.3 lists the various standards and recommended criteria for HCl, NO<sub>2</sub> and HNO<sub>3</sub>.

**Table 1.3 Comparison of Standards and Recommended Criteria**

Compound	ACGIH TLV	NIOSH REL OSHA PEL	IDLH	COT EEGL	COT SPEGL
HCl	5 ppm C	5 ppm C	50 ppm	100 ppm (10 m) 20 ppm (1 h)	1 ppm - 1 h 1 ppm - 24 h
NO <sub>2</sub>	3 ppm 5 ppm STEL	1 ppm STEL	20 ppm		1 ppm - 1 h
HNO <sub>3</sub>	2 ppm 4 ppm STEL	2 ppm 4 ppm STEL	25 ppm		

## **1.7 ACCEPTABLE RISK**

The Range Safety Requirements EWR 127-1, Section 1.4.1 refers to PL 60, pg. 1235 and notes on pg. 1-12 "From a safety standpoint, they [missiles] will be no more dangerous than conventional airplanes flying overhead." Section 1.4.1 prescribes the following risk criteria:

- $30 \times 10^{-6}$  for the general public (30 **casualties** in 1 million)
- $300 \times 10^{-6}$  for essential launch area personnel (300 **casualties** in 1 million)

EWR 127-1 notes: "The basic standard for the general public is not more than the risk voluntarily accepted in normal day-to-day activities." Appendix 1D was cited for further

information on acceptable risk criteria, which referenced a "Risk Commonality Acceptability Workshop, August 1990."

The Chemical Manufacturing Association (CMA) published guidance on risk communication, risk statistics and risk comparisons (Covello, 1988). This report noted that the risk of death from a falling aircraft in the U.S. was 1 in 10 million. A careful risk comparison requires knowledge about the data sources, assumptions, and other qualifiers. Those data cited are from 1980; the EWR 127-1 cites the WASH-1400 (NUREG/74/104) (1975) regulation which is older yet. The CMA reference also cited WASH-1400 which reported 1 person death per year in 100,000 from air travel.

In 1990, Congress passed the Clean Air Act Amendment (CAA) in response to the Bhopal, India incident. The CAAA mandated process safety management (PSM) under OSHA and risk management plans (RMP) under the US EPA for classified extremely hazardous substances (EHS) that exceeded threshold levels. Per 29 CFR 1910.119, Appendix A (57 FR 7847, March 4, 1992), PSM compliance are required for the following missile-related extremely hazardous substances when their reported threshold quantity are exceeded:

nitrogen tetraoxide	250 pounds (oxidizer)
hydrogen chloride	5,000 pounds (reaction product of solid propellant fuel/oxidizer)
nitrogen dioxide	250 pounds (reaction product of fuel/oxidizer)
nitric acid	500 pounds (reaction product of fuel/oxidizer)

The goal of PSM is to consistently reduce the risk to a level tolerated by the facilities workers, its management, surrounding communities, public, industry and governmental agencies. Tolerable risk is not an absolute number that can be fixed for all time but a relative standard that will vary over time and in different contexts. A general PSM trend has been a convergence of public opinion, governmental regulations, and industrial initiatives. Momentum in controlling such risk will continue and many chemical industries companies are setting standards well in excess of what is required (Stricoff in Kolluru, p. 8-47).

However, Stricoff noted that quantitative risk criteria could be established to provide some guidance on levels of acceptability and suggested two possible criteria as follows:

- Average individual **fatal** risk level for the public should be less than  $1 \times 10^{-6}$
- Maximum individual **fatal** risk for employees should be less than  $100 \times 10^{-6}$

These proposed values seem to be based on the EPA risk criteria for cancer, which may or may not be fatal. However, these proposed levels are 30 and 3 times lower, respectively than the general public and launch facility worker risk causalities criteria currently listed in EWR 127-1 (1995). Acceptable levels for cancer risk have been defined as one excess cancer in 1000 occupationally-exposed population and one excess cancer in 10,000 to 1,000,000 population for environmental exposures.

Of critical importance is the public tolerance of the missile launches and operations. The mere establishment of credible "safety levels" may not guarantee public tolerance. In potentially-charged climates, public perceptions are a reality. These perceptions can be frequently far from any scientific basis, but perceptions are so strongly held that scientific reality can not alter opinion. Until risk communication begins with addressing these public perceptions concurrently with scientific reality, there will be no improvement in the general understanding of relative risk and little acceptance of slight risk (Cox, 1988).

In summary, the acceptable risk criteria for missile operations may require a dialogue by the Air Force with, affected local communities, workers dealing with launch operations, appropriate regulatory agencies, and the public at large. It should be noted that California has established two programs to regulate chemical process safety: the Chemical Accident Prevention Program (1988) and the Process Safety Management of Acutely Hazardous Materials (1994). These programs overlap in some areas with the OSHA PSM rule and the US EPA RMP rule. It may be appropriate for the Air Force to assess whether it has requirements on missile operations for all three regulations before developing their response to minimize duplication of efforts. Regardless whether the Air Force must or must not comply with the PSM provisions, these established processes for chemical accident prevention and risk assessment procedures do set a precedence or useful framework.

## 2.0 PERSPECTIVES ON ASSESSING PULMONARY TOXICOPATHOLOGY

### 2.1 INHALATION DOSIMETRY

Dosimetry refers to the measurement or estimation of a quantity of a chemical absorbed by a target site and demonstrates exposure and response data that can be transformed to dose response relationships. The extrapolation of animal data to humans, requires knowledge of cell sensitivities, repair processes, cell specificity and defense systems which will vary considerably among different species. Metabolic rates, toxic mechanisms, genetic makeup, toxicokinetics and dynamics of the toxicant require evaluation. Unfortunately, the dosimetric process is much more advanced than the involved knowledge of quantitative relationships of species sensitivity so that the effort becomes a project in the art of extrapolation using the biomedical sciences. Text in this section is provided to allow the non-toxicologist insight to the biological effects reviews presented in sections 3, 4 and 5. Detailed toxicological information is provided in these sections to aid the peer review.

This review will focus on those effects that are produced from short term, low level exposures. This includes evaluation of toxicological dose and response data from normal and/or sensitive humans and from experimental laboratory animals. The objectives of a study, the diagnosis and assessments of resulting adverse health effects, the kinds of toxic effects or injury sustained by the respiratory tract airways and parenchymal tissue will be considered. Where possible, identification of organ dysfunction, and/or the accompanying change in physiology, cellular and/or biochemical endpoint, at differing exposure levels or duration will be identified and classified.

Ideally, a toxicological risk assessment of inhaled toxicants is a synthesis of an assessment of the biomedical data base of human and animal responses to various levels of some toxicant, a knowledge of mammalian physiological relationships integrated with expert judgment and common sense. In spite of the process, determination of the impact of acute airborne exposures on public health, to normal healthy humans or to sensitive sub-groups, from exposure to ambient levels of gas or aerosol rocket combustion products is filled with numerous obstacles that invite frustration.

Inhalation of airborne toxicants is the most significant route of exposure from the environment due to its immediate contact with and rapid absorption onto the large surface

area in the respiratory tissue involved in gas exchange. Any adverse effects may be significant due to its' subsequent rapid entry into the circulatory system and immediate distribution to the body.

Responses to inhaled dose presents a special set of problems. In addition to varying mammalian physiology parameters, the mechanics of airflow in the respiratory tract can drastically modify the absorbed dose. The approach to analysis of inhaled exposures requires answers, that may not be available, to a set of important questions. What is the relevant dose? Is it the neat chemical, or some metabolite causing response? How much is absorbed? Is it the amount of mass inhaled per unit of breath, the mass retained per body weight, the mass per body mass or per respiratory tract area which correlates to response? Other questions are generated during the evaluative process.

The response to an inhaled dose is a function of delivered dose and various values are reported for  $\text{NO}_2$  absorption along the respiratory tract. However, the reasons for such variability can be many; species specific variability in anatomy and the physiology of the respiratory passages, variance in experimental objectives or analytical and procedural methodology, methods for quantifying the delivered and/or absorbed dose and individual interpretation of the end point.

Exposures may produce lung injury with a single inhalation to some, while others may require larger dose or an unusually extended duration of exposure to insure that the dose initiates an adverse effects. Understanding these effects and predicting allowable exposures accurately is fraught with many other obstacles. To minimize these variations, scientists have proposed the use of various mathematical models (Dahl, 1990; Schlesinger, 1992). These mathematical models describe reactive gases, metabolizable vapors, nasal and/or lung uptake as a function of tissue metabolism, respiratory parameters and physico-chemical properties of the vapor. However, none provide ideal information on identifying gas uptake. The net effect testifies to the difficulty toxicologists have analyzing dose and corresponding responses by models, because the response is usually very limited and highly focused in its' applicability.

One dose model for  $\text{NO}_2$  predicts that in humans, gas is absorbed along the entire tracheobronchial tree and at the gas exchange units, with a majority of the tissue dose occurring at the transition zone, the junction of air ducts and alveoli. Lung morphometry

studies in rat, guinea pig, and rabbit suggest similar conclusions (Miller, 1982). However, beyond the transition zone, a rapid increase in surface area causes a fall in delivered dose and results in a 100 fold difference in peak dose at the tissue within the lung. What then, is the absorbed dose, in which tissue did it occur and which effects are relevant? Another major factor limiting accuracy or precision in extrapolation is the difference in absorption among species inhaling the toxicant. Comparison of interspecies anatomy demonstrates the significant differences that must be interpolated. Studies on casts of the comparative anatomy of the respiratory tract among a rat, a guinea pig, a beagle, and a monkey revealed the huge differences in nasal cavity surface area among different species (in cm<sup>2</sup>):

**Table 2.1 Comparison of Various Species Nasal Cavity Surface Area, (cm<sup>2</sup>).**

RAT	GUINEA PIG	BEAGLE	MONKEY	MAN
10.4	27.4	220.7	61.6	181*,

\*does not include sinuses only for man, (Schreider, 1986)

The beagle's nasal cavity has a large surface area which may even exceed that of humans for potential absorption, even though the typical human is 7-10 times larger. The effective absorbed dose is much greater and any physiological effects may be more intense in response or its effects recognized earlier due to the greater area of absorptive capacity within the smaller body volume and size. The greater concentration absorbed could lead to a conclusion of higher potency due to the significant response.

If we look at the rat nasal surface area, at 10.4 cm<sup>2</sup>, it is about 18 times smaller than the human nasal surface area. Yet the rat only weighs 250 gm. which is 280 times smaller than the standard 70 kg human. Balancing the disparity in nasal area and body size and volume leads to serious difficulty when extrapolating an animal response to some exposure level or dose.

Also, the nasal cavity of the rat is a 2.3 cm long tube, vertically sectioned with a maximum diameter of 9.6 mm in the tortuous turbinates, which has a large surface area for humidification. Several diameter gradients occur thereafter toward the trachea. The human nasal cavity has a length of 7-8 cm with the largest diameter of 40-45 mm. and several acute angles to be maneuvered during inspiratory flow. The rhesus monkey has a similar

anatomy to the humans, but shows considerable enlargement within the turbinates and the air makes a 90° downward turn from the oropharyngeal to the tracheal tissue. Thus, the diameter and tortuosity of the nasal cavity, the sinuses, the angle of bend in the nasopharynx, significantly affect the degree of impact, absorption and distribution of the toxicant. Interpolation becomes extremely complex.

Uptake in the upper respiratory tract of laboratory animals, is likewise highly variable, and ranges from 28% to 90 % of the volume inhaled. The subsequent uptake in the lungs was 36% to 90% of the amount entering the trachea, indicating that a large amount of the inhaled dose is removed by the respiratory tract (Schlesinger, 1992).

Tissue susceptibility and degree of response will determine the effects of any delivered dose. Yet, variability in interpreting any histopathological change as observed by different veterinary pathologists, and its' reinterpretation by non-pathologists, initiates a cascade of uncertainty factors that further confounds meaningful or effective interpretation. The histopathologist will categorize a response, based upon the variations in structural morphology relative to normal cell/tissue types, and a tissue response may be identified as a toxic effect due to an observed alteration. Although the interpretations of any alterations are important, the more important question that must be answered is, where and when does "physiological flexibility and adaptation" stop and an adverse response or effect begin ? Respiratory tract changes in tissue are described as altered but may merely reflect a physiological adaptation to a noxious insult that is a reversible response.

Squamous cell metaplasia (SQ. M.) is replacement of one adult type cell with another, in the epithelial tissue of the organ affected. It is an adaptive, reversible response to persistent stimuli (Rubin and Farber, 1988; Burger, 1989), indicating the tissue's reaction to a stimulus. SQ. M. occurs in animals housed on soiled bedding ( $\text{NH}_3$ ), or from Vitamin A deficiency or a viral infection or it may result from age dependent anatomic variation. Since as many as 10% of control populations may also show SQ. M. without dosing, SQ. M as an adverse response may be incorrect. Aggregation of alveolar macrophages, presence or concentration of various inflammatory cells, keratin structure and distribution of mucin cells are similar response situations that may have been described as an adverse response to some chemical agent (Cotran, 1995). Thus, the objective identification of an observed change and its' subsequent reinterpretation, could be an adaptive or a toxic response: The

outcome of any interpretation has a significant bias on risk characterization and development of allowable exposure levels.

The variability of cell types within respiratory tissue further adds to the dilemma of identifying an adverse effect and correlating exposure as a causative factor. The respiratory tract tissue is usually divided into pulmonary sub-divisions that include: 1.) naso-turbinate, 2.) laryngo-oropharyngeal, 3.) tracheobronchial and, 4.) the alveolar duct and sacs. These tissues are highly vascularized tissues, and are composed of forty different specialized cell types, that respond differently to inhaled toxicants. In addition to cell types, natural mechanisms will affect the potential for an adverse effect. In many cases, altered response of a rare type cell cannot be detected, but if detected, the change cannot be properly interpreted as to its significance.

The interrelationship of airborne exposures, absorbed dose, specific toxicant effects and the physiology, anatomy, and pathology of the respiratory tract is very complex. Of necessity, any interpretation of the data produces a partial picture of the true nature of any effects of the inhaled gas or aerosol. Details of species variability, extrapolation of data, meaningful interpretation of end points, and variability in response among experimental species and humans cause a great deal of difficulty when identifying toxic effects within different tissues at different exposures. Add, differing physical and chemical properties of inhaled particles, the aerodynamic particle size, and air flow characteristics, as well as the variability of dose and response interpretation becomes very difficult indeed.

Paracelsus, the first recognized scientific toxicologist concluded, rightfully so, that, "dose alone" is the ultimate criterion of concern in the matter of toxicity (Doull, 1994). None the less, particle deposition, biological susceptibility, as well as physical and chemical reactivity of the particle are all important in inhalation toxicology. Many different biological factors, the physical and chemical parameters of the toxicant, mechanics of airflow, toxicant entrainment and impaction, Brownian movement and deposition patterns, absorptive characteristics, etc., are all important factors that will contribute to understanding pulmonary toxicity. Minute variations in the respiratory tract can also dictate deposition of toxicant and ultimately the bioavailable or absorbed dose. This "bioavailable or absorbed dose" or that which finally reaches the target organ tissue to initiate some effect, may be more precise when describing toxicological effects to the respiratory tract. Rate of mass transport and absorption into the tissue may control toxic response and result in "dose-rate" implications.

Inhalation of intoxicating particles, can cause respiratory tract effects that may be immediate or latent and can be expressed as a frank clinical effect in a few hours or perhaps, many years later. The absorbed or bioavailable dose, the genetic makeup of the species used for testing, the numbers and sexes, the affect of living conditions, the level and duration of exposure, the health and/or susceptibility of the receptor, the distribution of toxicant along the respiratory tract, simultaneous multiple chemical exposures, workplace exposures, lifestyle and a host of other genetic and/or somatic factors can alter response. The final effect is so highly dependent upon these large number of variables and their interactions, that working individually or simultaneously, these parameters will significantly alter the possible response of any receptor and cause consternation.

## **2.2 TOXICOLOGICAL LEVEL OF EFFECTS**

Frequently, although not always, the results of exposure to a toxicant can be characterized by the type of response generated. These responses may be classified according to the degree of severity and the number of classes will depend upon the writer's viewpoint. However, it is a construct and schemes for classification are usually quite similar. The level of response classification for the purposes of this report are as follows:

- 1.) The No Observed Effect Level (NOEL) of exposure. This classification indicates that no observed physiological effects were observed at any dose or exposure level.
- 2.) The Lowest Observed Effect Level (LOEL). An observed effect that can be observed. It may be sensory or a mild surface irritation of the respiratory tract that is transient, generally lasts only a few hours, fails to alter organ function and resolves within 24 hours without any sequelae.
- 3.) The Lowest Observed Adverse Effect Level (LOAEL). A significant effect or injury occurring within the upper or lower airways, that affects normal organ function, may require medical treatment but is reversible and without any significant chronic effect on organ function.
- 4.) A Clinically Frank Adverse Effect Level (CFAEL), is an irreversible or chronic adverse effect, that alters normal organ function, due to its' affects on epithelial or parenchymal

tissue, the bronchioalveolar ducts or sacs and requires medical treatment. Emphysema or cancer may be such an effect.

5.) A final classification is, the Clinically Lethal Dose or Lethal Concentration ( $LD_n$  or  $LC_n$ ) that is fatal to  $n$  percent of dosed laboratory animals. It is used primarily to describe the fatalities to some level of toxicant developed in experimental laboratory animals.

As is evident from these divisions, distinctions between classes centers upon whether the insult caused a change that can alter normal organ function and whether the change was temporary or permanent.

## **2.3 DEFENSE MECHANISMS**

### **Mucociliary Movement**

The respiratory tract is well defended by physico-chemical characteristics and an effective, well designed cellular host defense system. Particle size and aerodynamics are the first to be considered to affect exposure dose. Particles of airborne aerosol or gas entrained in the airflow, are usually laminar. However, inhaled air entering the respiratory tract, passes the nares and enters the turbinates, where it makes two  $90^\circ$  turns before entering the tracheobronchial tree. What was laminar airflow becomes turbulent, due to the turbinates. The tortuosity of the turbinates initiate turbulent airflow and inhaled particles 10 microns in size, or larger, impact the mucous covering the epithelial tissue. These large particles cannot make rapid turns and will not reach the gas exchange units. They are amassed on the mucous surface layer for removal. The mucous surface solubilizes airborne materials and removes them during mucociliary flow, and is a major and significant barrier for removal of inhaled airborne materials.

As inhaled air continues over the larynx into the tracheobronchial tree, an additional mechanical barrier is at play. The tracheobronchial tree is defined by a bifurcation of the two major bronchi, by secondary cartilaginous bronchi and respiratory bronchioles. These tissues are lined with ciliated epithelium and also are covered with mucous, produced by mucous secreting cells, (e.g., goblet cells, brush cells and Clara cells [Types I/II], plus several other rare types). The cilia are bowed, and rhythmically move in an upward motion, to push mucous containing dissolved toxicants, from the deeper recesses of the lung toward the laryngopharyngeal area. The mucous is then removed by coughing and expectorating.

This is the second major defense barrier for removal of toxicants, usually particle size of about 1-5 microns in diameter.

The final tissue possibly impacted by the airflow is the gas exchange ducts and alveoli. Particles approximately 0.5-1.0 micron in diameter and residual unabsorbed gases may enter these tissues. The particles that are smaller than 0.5 microns are generally unaffected by the mechanics of airflow, and are controlled by Brownian forces. They may stay inside or move back out with the expiratory airflow. However, the air sacs are composed of various types of epithelial cells (alveolar type I, [gas exchange cells] alveolar type II, [storage of surfactant cells] and type III cells). They are thin to allow gas exchange and if affected, can alter pulmonary function.

### **Host Defense-Immune System**

The second important mechanism is the pulmonary cellular defense system, that normally resides in the pulmonary portion of the lungs. It also acts in concert with the immune host defense system. A toxicant stimulus, such as an airborne particle that affects the pulmonary tissue, causes response by alveolar macrophages. Alveolar macrophages (AM $\theta$ ) move freely about the airway, the lymphatics and blood vessels and play a host defense role to remove inhaled foreign particles, bacteria and the like, that helps to maintain the sterile nature of the lung. The process known as phagocytosis, includes ingestion of the foreign particle and release of reactive oxygen species (hydroxyl ion, singlet oxygen, peroxide, etc.) and very powerful oxidizing enzymes, that act to detoxify and/or destroy the foreign particle. However, oxidizing agents from the AM $\theta$ , can also spill into surrounding tissue and cause damage to healthy cells and tissue.

This response to a variety of insults and the phagocytic process may attract additional cells, called lymphocytes and polymorphonuclear leukocytes (polys). These cells further assist removal of these particles from the respiratory tract and help stem the insult. Polys contain powerful oxidizing enzymes much like the AM $\theta$  and whenever there is a large influx of polys, together with AM $\theta$ s, the potential for significant adverse effect increases. The dose of foreign particle determines the degree of defense response and the potential for an adverse effect, such as oxidation damage of tissue.

Lymphocytes are of two general types: T cells and B cells. T cells will mobilize to the site of injury or insult, and is responsible for delayed type hypersensitivity. They act in

concert with AM $\theta$  to defend the insult to the tissue. The B lymphocytes produce immunoglobulins, that along with many other duties, enhance AM $\theta$  activity and cytotoxicity that can be misdirected. Thus the response to any insult can be local and minor or local and large, depending on the insult and the body's response to the insult.

Interstitial spaces are composed of elastic fibers, collagen fibrils and fibroblasts that provide contractility and elasticity of lung tissue. Damage to these tissues via action by AM $\theta$ , polys and lymphocytes, during the inflammatory response process may be sufficient to cause distortion or fibrotic change, producing significant alterations in alveolar gas exchange capacity. Thus, the human response to any insult may initiate the pathological process which effects the terminal respiratory unit (alveolar epithelium, interstitium or vascular endothelium). If it is significant, the impact can affect gas exchange and alter function not only of the respiratory tract and organ, but of the entire organism.

## **2.4 CONTROLLED HUMAN EXPOSURES AND PULMONARY FUNCTION TESTS**

Pulmonary function tests are used to determine adverse effects of airborne toxicants and to identify whether there has been an alteration in the body's resistance to the flow of air. Any change that deviates well beyond the baseline or norms is considered an adverse effect and may indicate a condition of pulmonary dysfunction. Controlled human exposure studies serve as an important source of pulmonary toxicology data, particularly for a pollutant such as NO<sub>2</sub>. Folinsbee (1988) examined methodology and experimental design of these studies. Young males most often participate in such studies. They tend to be without allergies, any history of respiratory disease, are non-smokers, and able to perform required exercises; in some cases they are well conditioned athletes. Occasionally, young women, different racial groups, children, adolescents, elderly persons, and adults may be involved. Other subject groups that have been specifically studied include asthmatics, patients with allergies or chronic obstructive pulmonary disease (COPD), or smokers, and subjects that are otherwise healthy persons but have upper respiratory infections (cough, colds, flu, bronchitis etc.). The groups may be considered potentially "sensitive subjects", especially the asthmatics, COPD patients, children, and the elderly.

For individuals with existing lung disease and/or hyper-responsive airways, special consideration of any potential impact of a pollutant exposure is required. These individuals include healthy elderly people and those with limited pulmonary reserves, who may suffer ill

effect from an insult. There is a potential for greater physiological harm and /or pathological consequence in such receptors. Children are also in this group of special concern because their lungs are still growing and developing; the possibility of a long term impact on lung health may be much greater than for the mature adult lung.

Controlled exposures occur in a laboratory setting. The natural mode of exposure is unencumbered breathing within an exposure chamber. Other modes of exposure include face mask, hood, or mouthpiece exposures. A controlled exposure implies environmental variables such as the pollutant concentration, temperature, and humidity are carefully monitored and maintained at some specified level. In addition, duration of exposure and amount of activity during the exposure are closely regulated and carefully measured; volume of air respired affects exposure dose. In order to simulate an outdoor exposure where the subject is active, many exposure studies include some form of controlled exercise. However, exercise alone can have some important and confounding effects, particularly in the case of exercise-induced bronchoconstriction in asthmatics. Exercise by itself initiates significant changes in pulmonary function response or significant increments in airway resistance.

Exercise-induced bronchoconstriction is followed by a period of several hours during which asthmatics are less susceptible to bronchoconstriction (Edmunds, 1978). This refractive period could alter the subject's responsiveness to  $\text{NO}_2$  or other inhaled substances. Thus the point at which a determination is made can affect the results and any conclusions of increased adverse effects. The major determinants of the exposure "dose" of a pollutant are the concentration of pollutant, the duration of the exposure, and the volume of air breathed (specifically, the route, depth, and frequency of breathing) during the exposure. Information is, of course, necessary to determine the actual dose delivered to the various "target" regions of the respiratory tract (i.e., total respiratory uptake). Many of these considerations have been discussed in greater detail by Folinsbee (1988).

In human exposure studies, the methods used for assessment of effects primarily involve "noninvasive" procedures. Various pulmonary function tests such as spirometric measures of lung volumes, measures of resistance of lung or nasal airways, ventilation volume (volume of air inhaled into the lung per minute), breathing pattern (frequency and depth of breathing), and numerous other "breathing" tests have been utilized. These tests

provide useful information about some of the basic physiological functions of the lung. Certain tests provide information primarily about large airway function; these include: 1.) dynamic spirometry tests (e.g., forced expiratory volume in 1 second [FEV<sub>1</sub>], maximal and partial flow-volume curves [including those using gases of different densities such as helium], peak flow measurements, etc.); and 2.) specific airway resistance or conductance measurements (SR<sub>aw</sub>, SG<sub>aw</sub>). These standard pulmonary function tests are easily controlled, simple to administer, and may provide an overall index of lung function, with a relatively low coefficient of variation (CV). The standard CV is about 3% for FEV<sub>1</sub> and about 10 to 20% for SR<sub>aw</sub>. However, because NO<sub>2</sub> deposits primarily in peripheral airways, many of the above tests may not provide the necessary information to fully evaluate the effects of NO<sub>2</sub>. Other tests that may provide evidence of small airway dysfunction include multiple breath nitrogen washout tests, closing volume tests, aerosol deposition/distribution tests, density dependence of flow-volume curves (using gases of different densities such as helium), and frequency dependence of dynamic compliance. None are used routinely since these procedures to assess small airways function are not widely accepted.

Somewhat more invasive procedures have also been more utilized in recent years to determine human responses to air pollutant exposures, including pharmacological, airway inhalation challenge tests, measurements of pulmonary clearance of inhaled aerosols, bronchoalveolar lavage, nasal lavage, and arterial blood gas measurements. Airway inhalation challenge tests are used to evaluate the "responsiveness" of a subject's airway tissue to inhaled materials. Tissue responsiveness changes with alterations in a disease state, such as inflammation associated with asthma, viral respiratory infection, or as a result of damage to the airway caused by disease or insult from inhaled toxic or allergenic materials. Thus, a problem in evaluating changes in airway responsiveness, with respect to inhalation of air pollutants, is that the baseline level of responsiveness can be altered by other factors not associated with the exposure. In testing for airway tissue responsiveness, bronchoconstrictors (such as histamine, carbachol, or methacholine) are generally used to elicit the response. Other challenge tests involve the use of allergenic substances, exercise, hypertonic saline, or cold dry filtered air. Responses are usually measured by evaluating changes in spirometric function tests or in airway responsiveness after a challenge dose is administered. Usually, the test proceeds for some period of time, and

measurements are taken, until some target effect level is achieved (e.g., doubling of airway resistance). The airway responsiveness is then characterized by the change in dose required to achieve that level. Smaller levels of challenge materials indicate an increased level of sensitivity to the exposure agent. The procedures for administering and interpreting inhalation challenges are discussed and have been published by several authors (Cropp 1979; Fish, 1979; O'Byrne, 1982).

Pulmonary dysfunction can be characterized by conditions such as emphysema, chronic obstructive pulmonary disease or restrictive pulmonary disease, which are similar, but different. Restrictive disease is a pulmonary condition characterized by reduced expansion of lung parenchyma such that it resists air flow, and there is a decrease in lung volume. Adult Respiratory Disease (ARDs) or pneumoconiosis, the dust diseases, are examples of restrictive disease. An obstructive disease is characterized by increased resistance to airflow at any level of the respiratory tract, due to complete or partial obstruction. Bronchitis, bronchiolitis, emphysema and asthma are examples of obstructive diseases. A change in pulmonary values from baseline or when compared to norms suggests that a dysfunction may be present, and when present may also suggest that the airborne exposure may be a factor in causing the dysfunction. However, changes are seen in these tests that occur when just air is administered to normal or sensitive individuals add uncertainty to these responses.

## **2.5 Effects Of Exposures On Asthmatics**

An important issue in the evaluation of human clinical exposure studies involving asthmatics is the variability in response between, and within analytical laboratories. In the absence of significant differences in the exposure protocol or exposure dose, an explanation frequently invoked to explain the differences in response is that the characteristics or severity of the disease may differ from one subject group to another. An Expert Panel from the National Asthma Education Program of the National Heart Lung and Blood Institute, National Institutes of Health, defines asthma as a pulmonary disease with the following characteristics: (1) airway obstruction that is reversible (but not completely so in some patients) either spontaneously or with treatment; (2) airway inflammation; and (3) increased airway responsiveness to a variety of stimuli.

According to the National Institutes of Health ( in Schwartz et al., 1990) about 10 million people, or 4% of the population of the United States, have asthma. The prevalence is higher among African Americans, older (8 to 11 years old ) children, and urban residents (Schwartz et al., 1990). There is a broad range of severity of asthma ranging from mild to very severe. The common asthmatic symptoms include cough, wheezing, shortness of breath, chest tightness, and sputum production. A positive skin test is a response to sensitivity of common allergens and is a typical feature of asthma. The respiratory effects of asthma is characterized by an exaggerated bronchoconstrictor response to many physical changes (e.g., cold or dry air, exercise), chemical and pharmacological agents (e.g., histamine or methacholine).

Asthma is typically associated with airway inflammation and epithelial injury (Cotran et al., 1996). In addition to basic anthropometric information such as age, height, weight, gender, and race, other information may be useful in characterizing an asthmatic. In order to evaluate differences between subject populations from one study to another, useful information includes baseline lung function, frequency of asthma episodes, nonspecific bronchial responsiveness, reversibility of bronchoconstriction, types of medication and use, specific serum immunoglobulin E (IgE) levels, skin test responses, response to exercise challenge, duration of disease, and factors that precipitate or aggravate the disease. In most chamber exposures using asthmatics, the exposures are accompanied by moderate exercise. The potential for an increase in airway resistance or decline in lung volumes or forced expiratory flow caused by exercise alone is a very important covariant in pulmonary studies. Exercise, even if moderate, can induce some degree of increase in airway resistance, even in clean air at room temperature and relative humidity (RH), at 20 °C, 50 % RH. In order to determine the true effect of an air pollutant in exercising asthmatics, the response to exercise must be considered. Accordingly, in studies reviewed in this report, a control exposure to clean air or exercise must be considered in assessing response.

Asthmatics who participate in controlled human exposure studies typically have mild allergic asthma. In most cases, these individuals can be without medication temporarily or may discontinue medication for the brief periods of exposures when procedures are conducted in non-allergic seasons. Controlled human exposure studies that evaluated respiratory effects of NO<sub>2</sub> exposure of asthmatics are summarized in section 4.

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### **3.0 TOXICOPATHOLOGY OF HYDROGEN CHLORIDE (HCL) EXPOSURE**

This section of the report, as with the others, is a compilation and summary of the results of clinically controlled human studies and experimental animal studies that identify the changes in physiological processes as a response to inhaled hydrogen chloride (HCl) exposure. This report includes data from earlier studies, but focused on results from recent information of human studies. This direction is temporally appropriate, primarily because such studies appear to describe the effects of short term, low dose exposures. These exposure levels and their duration are closely allied to those associated with rocket emission products. Accordingly, they are related to earlier studies to provide support in any similarities of physiological responses identified during these HCl studies.

#### **3.1 STUDIES OF HCl EFFECTS IN ANIMALS**

##### **Review Of Early Studies**

The available database of human or animal exposures and their responses is limited. Nor are data demonstrating dose response, absorption, distribution, metabolism and excretion of inhaled exposures and any toxic effects or mechanisms well characterized.

The National Research Council (NRC, 1991) toxicology committees reviewed the toxicological effects of HCl and concluded, "the effects of exposure include, coughing, pain, inflammation, edema, and desquamation of the upper respiratory tract. High concentrations of HCl, can produce laryngeal or bronchial constriction and, closure of the glottis." Examination of the early studies indicate that the effects of low levels of exposure were not very severe, but high concentrations could be lethal. Malek and Alarie (1989) exposed exercise conditioned guinea pigs to 107, 140, 162 or 586 ppm HCl for 30 minutes. Animals exposed to 107 ppm for 30 minutes exhibited only mild irritation while animals exposed to the higher doses died at various times afterward, depending upon the exposure duration to the higher concentrations. The group exposed at 586 ppm died after only three minutes exposure, exhibiting severe respiratory irritation, gasping and an 80% decrease in respiratory frequency. Those effects were confirmed by Burleigh-Flayer (1985), who also reported corneal opacities, inflammatory changes, including alveolitis with congestion, hemorrhage with hyperplasia, and mild bronchitis.

Results of studies with mice, rats, guinea pigs, exposed to very high concentrations of HCl, (300 ppm to 1,586 ppm for from 30 min. to 6 hr.), demonstrate severe cellular

exfoliation, erosion, ulceration, necrosis of the nasal epithelium, ocular damage, and turbinate damage. Fatalities occurred after 5 minutes exposure at levels of 13,700 ppm in mice and at 41,000 ppm in rats (LC50s). The results of these studies indicate that mice are much more sensitive to the effects of HCl than are rats or guinea pigs. Data compiled by Einhorn (1975) indicate the effects among various animals from HCl exposure generated during the combustion of polymeric materials are in TABLE 3-1.

**TABLE 3-1 SUMMARY OF TOXIC EFFECTS OF HCl EXPOSURE AMONG EXPERIMENTAL ANIMALS**

<b>Conc. in ppm</b>	<b>Animal species</b>	<b>Toxicity of HCl on animals</b>
50	Monkey	Tolerable for 6 hr. daily
300	Guinea pigs	Mild corneal damage after 6 hr.
3200	Mice	No mortality after 5 min.
4300	Rabbits	Lung edema; death after 30 min.
13745	Mice	LD <sub>50</sub> = 5 min.
30000	Rats	No mortality in 5 min.
41000	Rats	LD <sub>50</sub> = 5 min.

The results in monkeys demonstrates tolerance to 50 ppm HCl for 6 hr. daily and is especially noteworthy due to the similarities of non-human primates with humans.

Subsequent studies using baboons who were exposed to 500, 5000, or 10,000 ppm HCl for 15 min. showed a high tolerance, as did the monkeys from the Einhorn report (1975). The non-human primates appear much less sensitive and responded much differently than the rodents (Kaplan, 1988). The baboons failed to show any significant changes in pulmonary baseline values from an exposure of 500 ppm level during experiments that required them to perform an escape task. Three days, and three months after exposure, respiratory frequency measurements using carbon dioxide (CO<sub>2</sub>) challenge did indicate an increase in respiratory frequency among the two highest (5000ppm and 10000ppm) exposed groups. The 500 ppm group failed to show any significant changes in respiratory frequency.

## EARLIER HUMAN STUDIES

A study given a primary position of importance as regards HCl exposed humans, has been cited (Henderson and Haggard, 1943) and can be found in many reports examining HCl toxicity and most recently, was cited in the NRC reports. Those results are summarized in Table 3-2 below on HCl toxicity.

TABLE 3-2 SUMMARY OF EFFECTS OF HUMAN EXPOSURES TO HCl

<u>EXPOSURE</u>	<u>DURATION</u>	<u>EFFECTS</u>	<u>REFERENCE</u>
<u>CONCENTRATION</u>	<u>TIME (MIN)</u>	<u>NOTED</u>	<u>CITED</u>
1000-2000 ppm	Brief	Dangerous for even short exposures.	Henderson and Haggard 1943
50 - 100 ppm	1 hour (60 min)	Tolerable	"
35 ppm	-	Throat irritation	"
10 ppm	Prolonged	No adverse affects.	"
1-5 ppm	-	Odor threshold	Heyroth, 1968

Source; NRC, 1991.

Combustion of polyvinyl chloride produces HCl that combined with water, produces hydrochloric acid aerosol that can cause damage to the mucous membranes (Einhorn, 1975). They investigated the toxic effects to humans from gas/smoke produced during combustion of chlorine containing polymeric materials producing Hydrogen chloride gas/aerosol; however, dose, and duration of exposure were not specified.

A significant report of human exposure showing the no observed effect level (NOEL) for humans is **10.0 ppm exposure for several hours duration** and a no effect level of 30-100 ppm for one hour, were reported from HCl exposures generated by burning plastics (Lefaux, 1968). The levels considered dangerous were 1/2-1 hr. at 1000 ppm and 30 minutes at 1300+ ppm HCl being rapidly fatal.

The reported acceptable industrial exposures for HCl at this time were ; 5 ppm (7 mg/M<sup>3</sup>) for the U.S., 15 ppm in Great Britain and 10 ppm in the USSR. App.1. 214. Lefaux, Source, The Practical Toxicology of Plastics, Ed. Rene Lefaux, 1968, Chem. Rubber Co., OH, Table on p. 207 and Appendix 1 p. 214.

## REVIEW OF RECENT ANIMAL STUDIES

There are very few, well controlled or well characterized studies elucidating the adverse effects related to various hydrogen chloride gas or aerosol exposures, either on humans or animals. Animal studies that have been conducted, examined, 1) acutely toxic levels of 5 and 30 minute HCl exposures and 2) whether the gas or aerosol exposure was of greater concern. Results of the study indicated that in mice and rats, the toxic LC50 concentration of HCl gas is comparable to the aerosol (see table 3-3). Further comparisons of the effects of gas versus aerosol exposure on mice, rabbits, and guinea pigs indicated that the toxic effects of HCl gas versus HCl aerosol (5  $\mu\text{m}$  diameters) were similar when the exposures were comparable. An exception was that the exposed rats were considerably more tolerant of the effects of exposure.

Table 3-3 SUMMARY OF ACUTE TOXICITY DOSE (LC50s) IN RATS AND MICE

LC50 $\Rightarrow$	5 minute ppm(mg/l)	30 minute ppm(mg/l)	60 minute (ppm)	Reference
<u>AEROSOL EXPOSURE</u>				
Rat	31,008(45.6)	5,666(8.3)	-	Darmer 1974.
Mouse	11, 238(16.5)	2,142(3.2)	-	"
<u>GAS VAPOR EXPOSURES</u>				
Rat	40,989ppm	4,701ppm	-	Darmer, 1974.
Mouse	13,745ppm	2,644ppm	-	"
Rat	----	----	3,124 ppm	
Vernon/West, 1974.				
Mouse	----	----	1,108 ppm	"
Adapted from Alarie, 1973.				

## Effects on The Upper Respiratory Tract

Qualitative results of studies on HCl exposures (Darmer, 1974) among laboratory animals demonstrate that the upper respiratory tract is most severely affected. Epithelial tissue in the nasal and tracheal passages was most damaged. Bloody discharges from the nose were the result of purulent bronchitis and caused labored and difficult breathing. Seven days post exposure, exposed animals were sacrificed and necropsied. Examination of the animals indicates tissue recovery from exposure was incomplete, based on consolidation of parenchymal and alveolar damage. The pathology of animals that died from acute exposures demonstrated moderate to severe alveolar emphysema, atelectasis, lung edema and occasional lung spotting. Acute toxic effects developing from 30 to 40 thousand ppm HCl exposures appears to have a potential to cause severe and chronic sequelae. Considering that such levels have not been reported as a significant response or toxicity in other studies suggests these levels of exposure should be further investigated.

### **Effects on the Nasal Cavity**

To determine where and the kind of lesions that are produced from HCl exposure, at the RD50 concentration, Buckley et al., (1984), dosed Swiss-Webster mice to induce lesions from the 309 ppm HCl exposure (the HCl-RD 50). The importance of this study is significant. It showed that the effects occur in the upper respiratory tract and they tend to occur when exposure duration is extended. These animals were exposed for 6 hours/day for 5 days, and exhibited lesions in the nasal cavity in a distinct anterior to posterior gradient. These lesions were primarily in the nasal cavity and none were found in the lower respiratory tract. The nasal lesions ranged from slight epithelial hypertrophy or hyperplasia, to epithelial erosion, ulceration and necrosis with variable inflammation of sub-epithelial tissues. These results also indicate that the probability of any lesions developing in the gas exchange units (alveolar ducts and alveoli) at 1/100<sup>th</sup> of the RD50 dose, which is 3 ppm, for an hour or less, appears to be a highly unlikely response. Extended duration of exposure (6 hr. x 5 days or 30 hr.) and the RD50 dose was required to initiate significant upper respiratory (nasal) tract lesions (Alarie, 1975). Exposure levels below 1/10<sup>th</sup> the RD50, but above 1/100<sup>th</sup> the RD50 appears to provide support for a value in that range, that is acceptable human exposure.

### Effects on Airway Ducts

The concentration of HCl vapor appears to control the breathing route, the pattern of depth, and the impact of any subsequent toxicological effects. This response was identified in studies among nose versus mouth breathing rats exposed to 1,300 ppm of HCl for 30 minutes. Animal inhalation exposures were controlled so that they ventilated either by a nose breathing tube (nose breathers - NB) or orally (mouth breathers - MB) where the nares were closed. One day after the exposure, the animals were sacrificed and tissues examined for injury. MB rats demonstrated a higher mortality and major disruption to tissue of the larger conducting airways, especially the trachea including epithelial, sub-mucosal, glandular and cartilage necrosis, and accumulation of inflammatory cells and exudates (Stavert et al., 1991). Mouth breathers inhaled to a greater depth and caused significant effects to the larger air ducts indicating that the pathological response, the magnitude of any injury from inhalation of HCl becomes a function not only of dose and duration, but also of breathing route.

Based upon the above values, Vernon and West suggest that Haber's Law,  $([C] \times \text{Time}[T]) = K$  may be applicable to HCl in both species of rodents in this range. However, as was previously shown by Darmer (1974), the rats were more variable in response and appear more tolerant to these exposures. A comparison is presented below and suggests that although the mice are more susceptible, the Haber values are in better agreement for mice than for rats.

TABLE 3-4 SUMMARY OF HABER VALUES FOR RATS AND MICE EXPOSED TO HCl

DURATION AND HCl CONCENTRATIONS FOR MICE AND RATS; HABER LAW			
AT	5 min. C x T	30 min. C x T	60 min C x T
Rats	204,490 ppm min.	141,030 ppm min.	187,440 ppm min.
Mice	68,750 "	79,320 "	66,480 "

## Correlation of Respiratory Rate and Exposures

Alarie (1973) studies of the effects of HCl exposure on mice described a characteristic increase in respiratory rate of mice during airborne chemical exposures. A concentration-response relationship was determined by animal testing for a large number of different airborne chemical exposures by Alarie and others (Alarie, 1973; Barrows., 1977; and Kane, 1978). These studies demonstrated relationships that were linear between the log of the concentration and a decrease in the respiratory rate of rats. Alarie found that a 50% decrease in respiratory rate of mice was an excellent predictor of how humans will react when exposed to similar concentrations. In mice, HCl sensory irritation occurs at 309 ppm (RD50). He states, the RD50 can induce an intolerable condition of sensory irritation and incapacitation in a human. However, at 1/10<sup>th</sup> the RD50 (30.0 ppm), humans might report a slight stinging or burning sensation to the eye, nose or throat; and at 1/100<sup>th</sup> of the RD50 (3.09 ppm) very slight or no sensory irritation would occur.

Subsequently, Barrow et al (1977), compared the sensory effects of Chlorine (Cl<sub>2</sub>) and HCl exposure on breathing rates among Swiss-Webster mice. These results were used to predict a threshold limit value (TLV). Based on the RD50, they predicted a TLV for Chlorine to be between 0.09 and 0.90 ppm. This compared favorably with the existing TLV of 1.0 ppm, which is at the upper limit of the range. This process provides support and a reasonable basis for predicting an TLV for hydrogen chloride (HCl). HCl sensory irritation causes an RD50 in mice of 309 ppm, and given Alarie's quantitation process, at 1/10<sup>th</sup> and 1/100<sup>th</sup> of the RD50, (3-31 ppm). Alarie's predicted TLV of 3.0 ppm was very close to the ACGIH TLV of 5.0 ppm.

The RD50 animal model is based on data that shows sensory irritation of the eyes, nose, and throat is caused by stimulation of the free endings of the trigeminal nerve located in the corneal, nasal and mucosal epithelium. Stimulation of the nerve endings evoke a burning, stinging or pungent sensation in man and is accompanied by various physiological responses. In exposed animals, the one consistent response was the pause in the expiratory phase of respiration that led to a decrease in respiratory rate.

The purpose of the RD50, and the 1/10<sup>th</sup> and 1/100<sup>th</sup> dose relationships was to evaluate the acute effects of airborne sensory irritants, and to establish a concentration range that would be useful for human exposure standards on an interim basis. These standards included developing TLV, EEGs, STEL and National Air Quality Standards for the

general population (Kane, 1979). Inherent in these guideline values is the concept that they represent the best estimate of acceptable exposure levels and they are based on current available data and technology.

Based upon these relationships, Kane (1979) proposed a five step relationship of toxicity, based on the RD50 and initiation of respiratory injury (see Table 3-5).

TABLE 3-5 SUMMARY:RELATIONSHIPS OF MOUSE RD50 TO HUMAN EFFECTS

CONC. FACTOR	HCl CONC. (IN PPM)	DESIGNATION OF TOXICITY EXPOSURE AMONG HUMANS.	REPORTED EFFECTS OF HCl
10xRD50	3000	Lethal	Lethal or severe injury to the respiratory tract.
RD50	309	Toxic	Intolerable sensory irritation. Injury from extended duration.
0.1xRD50	30	Effective	Definite, but tolerable. **
0.01xRD50	3	Ineffective	Minimal or no sensory irritation.
0.001xRD50	0.3	Safe.	Level of no effect.

Adapted from Table XIII, page 222, Kane et al., 1979.

\*\*An unpublished NAS/NRC report, Chlorine and HCl, by H. Hoyle, 1976 was cited in the Kane report that indicated no organic damage at 5.0 ppm, and an odor threshold of 1-5.0 ppm. These calculations suggest that 3.0 to 5.0 ppm, is a very safe exposure level and humans would appear to be unimpaired from such an exposure.

### Studies With Baboons

The baboon is a sophisticated model, very close to humans, that provides support for exposure levels that can reasonably be argued as acceptable for most human receptors. Examination of pulmonary functions among baboons exposed to 500, 5000 or 10,000 ppm of HCl for 15 minutes indicated a concentration related increase in frequency and minute volumes and a decrease in blood partial oxygen pressures (PaO<sub>2</sub>), (Kaplan et al., 1988). However, these affects were transient. Subsequent analysis of pulmonary parameters and functions, three days and three months after the exposures, failed to demonstrate any significant alterations indicating a rapidly reversible, non-chronic effect. In another study

using baboons, 1500 ppm HCl or higher was required to cause any significant respiratory toxicity among the baboons trained to perform escape functions, (Kaplan, 1985).

Federal agencies, (USEPA/FEMA/USDOT, 1987), using the level of concern (LOC) value, which is one tenth (1/10<sup>th</sup>) of the IDLH value, when based on the baboon data, would suggest a 150 ppm LOC, which is too high and could cause serious problems. However, using 1/100<sup>th</sup> of the 1500 ppm baboon value produces a fairly safe 15.0 ppm acceptable exposure, that might cause some mild transient sensory irritation. This level of exposure is reasonably close to and consistent with, the Henderson and Haggard (1943) data of 10.0 ppm. It suggests that 15.0 ppm, may be the dose that does not produce any adverse effects and does not appear to be unreasonable for a one hour exposure for a healthy individual.

### **3.2 STUDIES OF HCl EFFECTS IN HUMANS**

#### **A. Effects Of HCl On Normal Humans**

The study most frequently cited in health effects assessments of HCl was conducted by Henderson and Haggard, (1943). The data summary (reviewed above in Table 3-1) indicates exposure to 50-100 ppm HCl for one hr. was tolerable. Exposures to 35 ppm for some short time (unknown), produced minor throat irritation, and exposure to 10 ppm for a prolonged period failed to produce any adverse effects. The results produced by Heyroth (1963), is also mentioned in the NRC publication, indicating that 1-5 ppm is the odor threshold.

A case history, described by Finnegan and Hodson (1989), describes a case of prolonged hypoxemia after inhalation of an unknown amount of airborne HCl vapor. The inhalation duration was 15 minutes long and occurred due to an HCl release from a chemical factory some 100 meters distant. The vapor was supposedly contaminated with a small amount of phosphorous trichloride (PCl<sub>3</sub>) and water. However, no measurements were reported nor was there a description of how much was a "small" amount of PCl<sub>3</sub>. The patient suffered irritation to eyes, skin and respiratory tract, with subsequent episodes of respiratory distress. Lung function tests performed between 12 days and eight months after the accidental inhalation indicated the patients' functions were unremarkable and generally within normal limits. These data are of questionable value since no measurements were reported.

## B. Effects Of HCl On Asthmatics

Fine et al. (1987) studied the effects of HCl aerosol, unbuffered or buffered with glycine, on eight asthmatics. They measured change in specific airway resistance in order to determine the importance of pH, titratable acidity and chemical composition on the effects of exposure. Only one of the exposed eight asthmatics showed a change in airway resistance ( a 50% change) when inhaling unbuffered HCl. However, when inhaling HCl aerosol buffered with Glycine at pH 2, all eight subjects demonstrated a 50% or greater change in airway resistance. The authors concluded that Glycine buffer acted to sustain the Hydrogen ion concentration and increase the action, leading to adverse effects. They also realized that, titratable acidity, the Hydrogen ion concentration and the chemical concentration are important determinants that must be considered when considering the effects of acid gases/aerosols on asthmatics. Other studies, Stevens et al., (1992) have shown that humans breathing these gases/aerosols exhale ammonia ( $\text{NH}_3$ ). The level of  $\text{NH}_3$  produced may tend to neutralize the acidity and reconcile toxicity. The kidneys also use  $\text{NH}_4^+$  to attract  $\text{Cl}^-$  ion for excretory purposes and thus metabolic activity could alter these responses. The details of these relationships which, although not well characterized, may be part of a series of defense mechanisms that may contribute to modifying any sensory effects of exposure by sequestering or neutralizing  $\text{H}^+$  ion.

The most recent, and convincing evidence for a lack of any significant effects from low level HCl exposure among asthmatics is provided by Stevens et al. (1992). Ten mildly asthmatic patients (five male, five female) were exposed to a mean HCl concentration of 1.84 ppm (exposure was  $1.84 \pm \text{ s. d. } 0.21 \text{ ppm}$ ) for 45 minutes, with two 15 minute periods of exercise and one rest period of 15 minutes. Exercise increases the depth of respiration and increases the total HCl dose inhaled. It should cause a deeper level of airflow and penetration into the alveolar ducts and sacs. No significant adverse effects to pulmonary function were identified from these exposure concentrations and duration;  $\text{NH}_3$  levels were increased after the exposures. The asthmatic subjects showed no adverse respiratory health effects of inhalation to these concentrations of HCl. Oral  $\text{NH}_3$  levels were also recorded with pulmonary function examinations and tests. Ammonia present in exhalations could neutralize the acidity of the HCl; However, activities that contribute to this event are presently unknown and should be examined further. In a personal telephone conversation

with Professor Stevens, it was concluded that a 2.0 ppm exposure among asthmatics is unlikely to produce any adverse effects, even if exposure duration was extended to one hour.

**TABLE 3-6 SUMMARY:ACUTE EFFECTS OF HCI ON ANIMALS AND HUMANS**

Concentration ppm	Exposure Time	Haber No. ppm x min.	Species	Response	References
309	6 hr./day x 5 days	1800.	Swiss-Webster Mice	Sensory irritation, nasal hyperplasia, necrosis	Barrow et al. 1986 Buckley et al. 1984
50 +higher 201 480	10 min.	2010.	Swiss Webster Mice	Sensory irritation at levels above 50 ppm. Ocular damage @480 ppm	Barrow et al., 1979
1300	30 min.	39,000	Rats(mouth breathers)	Trachea/airduct tissue necrosis. Exudate and inflammation.	Stavert et al. 1991
10, 20, 50	6 hr/d x 5 d/wk x 90 days		Rats and Mice	Minimal mild rhinitis, cheilitis; mild change, Turbinates affected. All reversible.	Toxigenics. 1983. 1985 (From NRC, 1991).
31,000.	5 min.	155,000	Rats	LC50	Darmer et al. 1974
5,666	30 min	169,980	Rats	LC50	"
3,124	60 min	187,440	Rats	LC50	Vernon and West, 1974
11,238	5 min	56,190	Mice	LC50	Darmer et al., 1974
2,142	30 min	64,260	Mice	LC50	"
1,108	60 min	66,480	Mice	LC50	Vernon and West, 1974
500	15 min	7,500	Baboons	30%↑ resp. rate. No sig fx 3d/3mo. post exp.	Kaplan, et al., 1987
5,000	15 min	75,000	"	50%↑ resp. rate. No sig fx	"
10,000	15 min	150,000	"	100%↑ resp. rate, PaO <sub>2</sub> ↓	"
1500	75 min		Baboons	Adverse resp. fx	Kaplan, 1988
107	30 min.	3,210.	Guinea pig (exercising)	Mild irritation	Malek and Alarie, 1989
>140	16 min.	840.0	"	Incapacitated in 16 min.	"
162	1.3 min.	240.6	"	80%↓ resp. rate.	"
586	0.6 min.	351.6.	"	dead after 3 min.	"
320	30 min.	960.0	Guinea pig		"

**TABLE 3-7 SUMMARY OF TOXIC EFFECTS OF HCl IN HUMANS**

Reproduced from NAS/NRC, 1986

EXPOSURE (ppm.)	EXPOSURE DURATION	EFFECTS OR COMMENTS	REFERENCE
1000-2000	"Brief"	Dangerous for short exposure	Henderson, 1943
91	--	Median Conc. To detect odor	Henderson, 1943
50-100	1 hour	Max. tolerable conc.	Henderson, 1943
10-50	Few hours	Max. tolerable conc.	Henderson, 1943
35	--	Irritation of throat after a "short" exp.	Henderson, 1943
10	Prolonged	No adverse effects	Henderson, 1943
10	--	Odor threshold	Leondardos, 1969
1-5	--	Odor threshold	Heyroth, 1963

**TABLE 3-8 SUMMARY OF EFFECTS OF HCl ON ASTHMATICS**

Concentration	Duration	Species Tested	Effects Noted	Reference
Glycine Buffered HCl, conc. unknown	unknown	Human Asthmatics	8/8 had 50% ↓ airway resistance	Fine et al., 1987
Unbuffered HCl, conc. unknown.	Unknown	Human Asthmatics	1/8 had 50% ↓ airway resistance	"
2.0 ppm	45 min. (w/ 30min. exercise)	Human Asthmatics	No effects observed	Stevens et al., 1992
unknown	unknown	Human	Irritation of eyes, skin, and respiratory tract; Hypoxemia. Lung function normal.	Finnegan and Hodson, 1989.

### **3.3 PRIOR RECOMMENDATIONS FOR AIRBORNE LIMITS**

There are several recommended values for allowable exposures to HCl, that are dependent upon the duration of exposure. In 1961, the National Academy of Sciences-National Research Council (NAS/NRC), Committee on Toxicology (COT) convened a meeting to consider a request by the US Air Force (USAF) to recommend short term "Emergency Tolerance Limits" for jet plane emissions. Subsequently, COT prepared recommendations for emergency exposure limits (EEL). The EEL subsequently became known as the Emergency Exposure Guideline Limits (EEGL), and was a ceiling limit, for a single emergency exposure duration. The EEGL was developed for different duration, beginning with the shortest exposure anticipated, 10-15 min. and working up to a maximum of 24.0 hr. (NAS/NRC, 1986). It was developed based on toxicity information and meant to prevent irreversible harm, including cancer, and is based on the assumption that a complete recovery will follow the exposure. The EEGL is for a single substance only, and does not address exposure to a mixture.

The NAS/NRC guidance also recommends other limits and advisory information. The SPEGL is defined as a suitable concentration for an unpredicted single, short term, emergency exposure of the general public, and considers a wide range of susceptibility of the general public, including sensitive populations (fetuses, children, the aged, ill and debilitated, and reproductive capacity).

The Continuous Exposure Guidance Limits (CEGL) is a ceiling value intended to avoid adverse health effects, immediate or delayed, and to avoid degradation in performance of military activity capability, even after 90 days of exposure for both on and off launch site, receptors. Prolonged exposure (24 hr. or greater) would be acceptable in this case as opposed to the EEGL which is an emergency exposure. Although regulatory agencies may consider the NAS/NRC information, these guidance limits are not regulatory limits: those issues require consideration of different exposure levels and duration, variable populations, multiple exposure sources, technical feasibility, risk benefit relationships, and other health/safety considerations. Previously developed guidance levels of acceptable public airborne exposures to hydrogen chloride (HCl) recommended by the NAS/NRC are presented below. (NAS/NRC, Vol. 7, 1986).

TABLE 3-9 PRIOR NAS/NRC RECOMMENDED HCl EXPOSURE GUIDELINES

<u>HCl EXPOSURES,* (in parts per million)</u>					
<b>EXPOSURE</b>					
<u>DURATION</u>	<u>1965</u>	<u>1971</u>	<u>1972</u>	<u>1977</u>	<u>1986</u>
10 min.	30 EEL	7 PEL/4 STPL	--	100 EEL	100EEGL
30 min.	20 EEL	3 PEL/2STPL	--	50 EEL	--
60 min.	10 EEL	3 PEL2 STPL	5EEL	20EEGL	20EEGL(1 SPEGL)

Data from, EEGLs and CEGLs For Selected Airborne Contaminants, Vol. 7, NAS/NRC, 1987.

### 3.4 DISCUSSION

There is some consistency, although they not highly correlated, among the reports studying the level of HCl exposure and effects of HCl exposure to normal or asthmatic humans and in experimental animals. Recent as well as earlier data of human exposures, normal and asthmatics, suggest that the animal data is informative and consistent among most species. The mouse appears very sensitive, but the rat is not as much so. However, the baboon data appears to be well suited for extrapolation to human exposure levels, and that low levels of exposure is not likely to produce a severe response nor is it very irritating. The indications from these exposures and responses suggest that at some level, the HCl is soluble in the mucous, and the effect is sensory. Surpassing that low level or extending the duration irritates the respiratory tract that could be of concern. However, sufficient data to establish precisely what that value may be is uncertain.

LeFaux's studies indicate no effect in humans from 10 ppm HCl for several hours, nor were there any effects in humans from 50-100 ppm for one hour. Somewhere in between 10 and 50 ppm may lie the acceptable exposure level. The report by LeFaux, indicates no effects from levels of 30-100 ppm and those doses failed to cause any pulmonary dysfunction among normal humans. The experimental modeling and dosimetry studies suggest that if there were impacts from HCl exposures, effects would most likely be seen in the nasopharyngeal region of the upper respiratory tract.

The Stevens group of asthmatics exposed to HCl demonstrates that a 2.0 ppm exposure for 45 minutes fails to elicit an adverse response, either in airway reduction or in lung function. The database for effects on asthmatics is weak. However, considering the asthmatic is a sensitive receptor, and did not respond to 2.0 ppm, suggests they may not be very sensitive to HCl. However, the preponderance of data suggests that there is a wide variability in response among the sensitive responders, and it is quite impossible to determine who would be the likely responders from any exposures. Therefore, the exposure data among asthmatics indicates that of 3.0 ppm does not appear able to provoke airway hyper-responsiveness. There are no data that indicate children, the aged or infirm, would be affected by HCl exposure and an acceptable level of exposure cannot be projected. Additional data will be needed on subjects with pulmonary dysfunction in order to determine any acceptable levels. Those data are presently unavailable and the available results by Stevens et al. (1992), although highly significant, is too small a population to draw significant inferences for the population.

Baboons do not demonstrate any chronic sequelae or adverse pulmonary effects from a 500 ppm exposure for several minutes duration. However the effects of such a short duration are difficult to interpret for extrapolation purposes. At 1500 ppm, baboon respiratory tissue was irritated. In humans, levels of 20 -30 ppm exposure, authors concluded there was a mild, slight or no effect, and was also without any sequelae. Thus at 1/100<sup>th</sup> of the 1500 ppm level in baboons, 15.0 ppm appears to be an acceptable level, consistent with other data that would be considered not to provoke any significant effects.

Guinea pigs fail to elicit any significant effects at 680 ppm for 30 minutes. The NRC recommended a two min. EEGL of 250 ppm, that was likely to cause some reversible effects and concurred with a 60 min. ceiling of 15 ppm.

Based upon the animal and human data examined and the effects noted, however, the following conclusions can be stated with a reasonable amount of medical certainty. HCl can be a relatively potent irritant to the respiratory tract of humans. However, the effects on mice appear to be excessive and they may be overly sensitive to HCl exposure data; this data is unlikely to be very meaningful and a rat appears to be a more suitable model. However, it too responds differently. The baboon data, used in concert with human information, appears to be the ideal for decision making and to support short term exposure levels.

## **CONCLUSION.**

Based upon human and primate animal data (LeFaux, 1968; Henderson and Haggard, 1943; and Kaplan, 1988) it appears that an infrequent, occasional exposure to 15.0 ppm HCl or less, of one hour or less, does not appear likely to cause any significant adverse, chronic or irreversible harm to a healthy human. Exposure may initiate a mild sensory irritation in some human receptors, however, it would most probably be transient, and only mildly discomforting: Irreversible effects appear highly unlikely. Although there may be some who would experience these effects, they are not likely to cause any permanent sequelae. There may be higher exposure levels that are without toxic effects, the present data do not support establishing a higher level due to uncertainty.

## **4.0 HEALTH EFFECTS OF NITROGEN OXIDES (NO<sub>x</sub>) EXPOSURE**

This section is a compilation and summary of the results and observations of clinically controlled human studies and experimental animal studies describing changes in physiological processes as a response to nitrogen dioxide exposure. This section includes data from prior studies and reviews but focuses on more recent human studies that are generally four hours or less exposure duration. This direction is appropriate, primarily because those studies seek to measure responses and/or effects of short term, low dose exposures. These exposure levels and duration are similar to the products expected from rocket emissions.

### **4.1 ANIMAL STUDIES OF EFFECTS OF NO<sub>2</sub> EXPOSURES**

In attempting to understand and correlate the exposures and responses of NO<sub>2</sub>, Folinsbee (1992a) assessed many studies. He hoped to identify the combined impact toward the meaning of the human and animal studies, but met with several obstacles. Each study varied in the important parameter measured, and it becomes very difficult to compare changes among different end points found in human and animal studies. Variability in study methodology, accuracy of NO<sub>2</sub> concentrations, duration of exposure, ventilation rates, lack of accurate dose-response data, animal housing conditions, varying physiological end points and the interpretation or meaning of these end points all contribute to the impractical nature of a comparison. None the less, the available refereed studies have been examined and included in this report, since these reports do provide information on the important relationships that were developed.

#### **Respiratory Tract Toxicity and Response Mechanics**

Nitrogen oxides can be absorbed along the entire respiratory tract, but primarily affect the lower respiratory tract alveolar ducts and terminal bronchioles. Any adverse response depends upon exposure concentrations, dose inhaled and the organism's susceptibility. Effects of acute exposure generally can be characterized by severe pulmonary symptoms after a symptom free interval. However, exposure may not always elicit a significant airway response, even among sensitive asthmatic receptors. Tissue morphology studies in exposed animals show that the transitional zone between the terminal bronchiole and the alveolar duct appears to be the most sensitive tissue to exposures from nitrogen oxides.

Alveolar effects tend to develop after exposure to high levels of NO<sub>2</sub> or from exposures of long duration (Sandstroem, 1991).

In several animal studies of NO<sub>2</sub> exposures, the presence of increased numbers of neutrophils, macrophages, lymphocytes and mast cells, in bronchioalveolar lavage fluid, indicate that the inflammatory response occurs when sufficient stimulus is provided. An immune response may occur if an antigenic substance is present. These responses are not in and of themselves adverse, but reflects typical, normal mammalian physiological activity to an irritant or noxious stimulus.

### **Effects of NO<sub>2</sub> Exposure on Rat Pulmonary Function**

Several studies reported altered pulmonary responses occurring from longer duration NO<sub>2</sub> exposures. However, the data are important, since exposures do not always provide the same responses. . Laboratory rats exposed to 6.0 or 12.0 ppm NO<sub>2</sub> for two hours with and without exercise, were subsequently examined for changes in lung function, ventilation rates (V<sub>r</sub>), and tissue morphology, (Bhalla et al., 1987). The exposure among non-exercising animals failed to induce any significant changes in permeability in either tracheal or bronchioalveolar (BA) zones when compared to controls. However, adding exercise to the exposed animals for two hours produced a significant increase in tracheal and BA permeability when measured one hour after exposure. There were no changes detected from baseline when measured at 24 or 48 hours post exposure. The exercise increased V<sub>r</sub>, causing deeper inhalation and subsequent penetration of the chemical stimulus not seen during resting exposures.

Gelzleichter et al. (1992) also studied the effects of NO<sub>2</sub> concentration on rat lungs in order to determine whether the effect was cumulative or a function of dose rate. By using a constant exposure level of two environmental gases, ozone and nitrogen dioxide, they were testing the validity of Haber's Law in response to a constant exposure. The total NO<sub>2</sub> exposures were 259.2 ppm for various periods of duration over three days. On each of the three days, different animal groups were exposed to four different concentration-duration values of NO<sub>2</sub>. The exposure duration was either 24 hour @ 3.6 ppm, 12 hour @ 7.2 ppm, 8 hour @ 10.8 ppm or, 6 hour @ 14.4 ppm,. Each exposure was 259.2 ppm x min. The same protocol was used for the ozone exposures. The results indicate that the cumulative dose from 24 hour a day exposures produced the least severe lung damage, as measured

by amounts of lavaged protein and recovered epithelial cells. The animals exposed for shorter duration showed significantly higher response in terms of lung damage, indicated by the significantly higher levels of Polys and lavaged proteins. The groups exposed to the highest levels, 10.8 ppm or 14.4 ppm NO<sub>2</sub>, showed yet significantly higher values for Polys and this response appears to be purely a function of dose. The combined exposure to ozone and nitrogen dioxide, showed deviations from Haber's Law, since the concurrent exposure to ozone and nitrogen dioxide, initiated significant synergistic effects that were not seen when the exposures were sequential. These data suggest that further laboratory study will be required to determine the effects of multiple chemical exposures from rocket emissions.

### **Histological and Biochemical Effects of NO<sub>2</sub> on Rat Lung**

The most sensitive indicators of lung injury are the cells found in bronchioalveolar lavage (BALc), especially the presence of polys, of protein and albumin (Alb), or angiotensin converting enzyme (ACE), and  $\beta$ -glucuronidase ( $\beta$ -G-ase) activity. In order to determine what histological and biochemical responses can develop from longer duration exposures, Meulenbelt et al. (1992) exposed rats to acute doses of either; 75, 125 or 175 ppm of NO<sub>2</sub> for 10 minutes. Dose dependent changes were found in several of the measured parameters. At the 175 ppm level, protein and albumin and ACE concentrations showed a hundred fold increase while  $\beta$ -G-ase showed a 10 fold increase. The changes indicate the pulmonary microvascular system became permeable. However, it is unknown whether this is an adverse effect or how significant the change. Upon further analysis, it was found that levels of Glucose-6-Phosphate (G6P), glutathione peroxidase (GP-ase) and  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), were the most practical parameters that could be measured since they were indicators determining the degree of tissue repair; measurement shows that tissue repair was complete in seven days.

Tissue biochemistry is significantly altered from 40 ppm NO<sub>2</sub> exposure for four hours. It was indicated by phospholipid peroxidation in the trachea and bronchial epithelial tissues in spite of high levels of Vitamin E antioxidant (Cavanagh and Morris, 1987).

However, the exposure concentrations are significantly higher and of greater duration than may realistically be expected to exist in the environment.

### **Effects of NO<sub>2</sub> on Mouse Lung**

Studies in mice show similar results, especially during concomitant exposure. Exposure to silica particulate followed by NO<sub>2</sub> exposure shows an increase in bronchioalveolar lavage polys, and indicates a significant response, since the vascular migration of Polys correlates with tissue permeability (Vetrano et al., 1992). The degree of influx of the Polys suggests that the exposure has initiated vascular permeability and again is considered an indicator of a significant response. This response is considered significant since Polys produce inflammatory mediators that can cause tissue damage.

## **4.2 STUDIES OF NO<sub>2</sub> EFFECTS IN HUMANS**

This section generally focuses on human studies conducted after 1990, however several earlier studies, some animal and some human, have been included. They are important because the information and their responses provide support to the physiological responses identified among the recent clinical studies on humans.

### **Epidemiology Studies**

Epidemiological studies have not been widely reviewed. The reports tend to produce mixed or inconclusive results in attempting to link exposures from NO<sub>2</sub> to adverse effects. In most cases it is due to a lack of accurate exposure data, or confounding variables that cannot be accurately identified or unaccounted for, and perhaps manipulation of data by sophisticated statistical evaluations that may produce added uncertainty to the significance of the data. Accordingly, the physiological response data in humans, at acute low level exposure conditions are given prime consideration.

### **Controlled Studies Of Human Exposures To Nitrogen Oxides**

This review is concerned with the potential for adverse effects from short term human exposures from the low levels of NO<sub>2</sub> generated by rocket emissions. Accordingly, this effort reviews earlier studies but focuses on recent and clinically relevant, human studies and especially those that are controlled. The responses from these studies have greater import and include a composite of measured end points of lung function and the

inflammatory response cells and/or products that identify changes occurring among receptors. These studies provide measured physiological responses observed over a narrow range of NO<sub>2</sub> exposures and duration which are of prime importance when considering public exposures. Although nitric acid was also studied, the known effects of nitric acid (HNO<sub>3</sub>) inhalation are discussed later in section five. The toxic effects of nitric acid requires further study, since NO<sub>x</sub> species are rapidly converted to nitric acid. Only recently has this compound been studied and the information of dose and effect, is sparse.

Controlled human exposure studies of NO<sub>2</sub> deal with low level and short term exposures as compared. A major benefit to reviewing these studies is that they evaluate the effects of fairly short-term (typically <2 hours) NO<sub>2</sub> exposures. Also, they provide important information by consideration of the time course of responses and pattern of exposure in these controlled human exposure studies. However valuable these controlled studies, there is a confounding factor that influences any conclusions of doses and effects. There is the widespread occurrence of NO<sub>2</sub> levels not only out of doors, but also in indoor environments. They occur simultaneously, and may predispose humans to become sensitized or even to raise the threshold. These unmeasured exposures cause great deal of difficulty in developing any potential conclusions of an adverse health effect from NO<sub>2</sub> exposure on human health. Ambient levels of nitrogen oxides, especially in California may be a significant confounding factor.

Dosimetry modeling and animal histological studies indicate that the impact of NO<sub>2</sub>'s effects tend to be seen primarily in the small airways and in the gas exchange regions of the lung. Accordingly, the tests that reflect possible relationships in this region of the respiratory tract are of particular interest in evaluating the effects of NO<sub>2</sub>.

As a group, asthmatics appear to be more responsive than healthy subjects to airway challenge. The differences in responsiveness between healthy and asthmatic humans can be several orders of magnitude (up to 100-fold) (O'Connor et al., 1987). Nevertheless, there is considerable overlap between the more responsive healthy subjects and the less responsive (histamine challenged) asthmatics (Pattemore et al., 1990). Airway tissue responsiveness to methacholine appears to be more effective than airway responsiveness to histamine when differentiating asthmatic subjects, although responses to bronchoconstrictors are well correlated ( $r = 0.70$ ) (Chatham et al., 1982). Unfortunately, because of the number of different challenging or provocative agents used in airway

challenge, and the variety of administration methodologies, it is difficult to compare these responses among laboratories or among different animals in a quantitative manner. Thus, it is not useful to suggest standard ranges of responsiveness for either healthy or asthmatic subjects.

Tests of pulmonary clearance of inhaled aerosols are used to assess the efficacy of the mucociliary clearance mechanism and to estimate pulmonary epithelial permeability. Typically, specific size range of a radioactive labeled test aerosol, that deposits in the region of interest is administered via inhalation. External scintillation detectors are used to detect remaining labeled test aerosol at various times after the initial deposition. The usefulness of this methodology is discussed in Clarke and Pavia (1980) and Raabe (1982). The rate of clearance of radiolabeled aerosols has been found useful for estimating epithelial permeability, using technetium-labeled diethylene triamine penta-acetate (Pentetate), (Nolop et al., 1987).

Bronchioalveolar lavage techniques are used for identifying cell populations in pulmonary fluid after clinical exposure to several different pollutants. In this procedure, a fiberoptic bronchoscope is passed into the airways and wedged in a subsegmental bronchus, where sterile buffered saline is used to wash out free cells and airway secretions from some segment of the airway (Reynolds, 1987). The resulting lavage fluid may be analyzed for various chemical mediators or reaction products, numbers and types of cells, and functions of some lung cells.

There are a number of limitations to the use of controlled exposures and clinical studies, whether in humans or among animals. Experimental animal models are frequently genetically pure strains and they reduce the expected variance of biological response seen among humans. Human heterogeneity produces a wide range of responses to a variety of physiological and pathological stimuli. This variability and the small sample numbers limit the extent to which the data can be generalized to the population as a whole or to certain sensitive segments of the population. The small sample size may limit the interpretation of the study, especially when the results are negative. Any study that fails to finds an effect of some treatment ( $\text{NO}_2$ ) in a small sample size, cannot be used statistically detect an adverse effect. If it is present, it is not treated with a high degree of confidence. This must be kept in mind in interpreting the results of human exposure studies with a small number of subjects.

Investigators have reported a wide variation in responsiveness of asthmatics to NO<sub>2</sub>, which may be partially attributed to intrinsic variation in response as well as variation in exposure variables. In addition, place of residence, season of the year, and indoor home environment may all be determinants of the asthmatic's response to NO<sub>2</sub>. Controlled human exposure studies are ethically limited to acute or sub-chronic and fully reversible functional, symptomatic and/or asymptomatic responses. This may limit the magnitude of expected responses and the statistical significance of responses in studies with small numbers. Since exposures seldom last longer than one or two weeks for up to eight hours per day, these data are useful in evaluation of short-term, but not in acute very short term, exposures to NO<sub>2</sub>-induced health effects.

True simulation of ambient conditions, given the number of potential pollutants and the variety of possible combinations, is not a realistic goal for controlled human exposure studies. For example, the typical temporal pattern of ambient concentrations is seldom duplicated in controlled exposure studies. Simple chemical mixtures can be evaluated to determine either additive or synergistic effects. Discussion of the design considerations for human clinical studies are presented by Bates et al. (1970), Hackney et al. (1975a), and Folinsbee (1988) and have been the subject of symposia. Controlled exposure studies of humans deal exclusively with acute or sub-chronic exposures, so that the applicability of these data are limited to short-term exposure effects and are of limited usefulness in assessing the effects of chronic NO<sub>2</sub> exposures. Many additional studies on the effects of NO<sub>2</sub> on healthy subjects are available since the 1982 EPA document, Air Quality Criteria for Oxides of Nitrogen Document was prepared. New studies of the effects of NO<sub>2</sub> on individuals with pulmonary disease (asthma and COPD) were published, reducing the critical lack of information (U.S. Environmental Protection Agency, 1992). The data base concerning NO<sub>2</sub> effects in sensitive subjects still requires concentration-response studies in sensitive asthmatics or COPD subjects, additional and specific information concerning the inflammatory response to NO<sub>2</sub> inhalation, and the mobility and accumulation of inflammatory cells, together with further detailed examination of NO<sub>2</sub> effects on infectivity of pathogenic microorganisms in humans. Only one study was reviewed on pulmonary epithelial permeability or mucociliary clearance effects of NO<sub>2</sub> in humans. One of the more important observations in studies of NO<sub>2</sub>-exposed animals is that exposure increases susceptibility to viral and bacterial infections due to impaired host-defense mechanisms. These studies

have provided a basis for several recent investigations of human immune host defenses after NO<sub>2</sub> exposure. Studies have utilized both in vitro exposure of cultured human cells (e.g., macrophages) and in vivo exposures of human subjects.

Recently published reports generally support prior conclusions regarding the effects of NO<sub>2</sub> exposure on healthy young adults. Several studies have reported the specific effect of NO<sub>2</sub> on cardiopulmonary function in healthy adults while another group of studies indicate possible adverse effects of pollutant mixtures of NO<sub>2</sub> in ambient air. Time constraints preclude further assessment of these studies.

Studies examining the specific effects of NO<sub>2</sub> in healthy subjects support earlier conclusions described by the USEPA, (1982), since they demonstrate a consistent absence of NO<sub>2</sub> effects on lung function at concentrations between 0.3 and 0.6 ppm (Adams et al., 1987; Drechsler-Parks et al., 1987; Drechsler-Parks, 1987). Studies have also been published in which NO<sub>2</sub> was a component of an ambient oxidant air mixture (Avol et al., 1983, 1985a, 1987; Linn et al., 1980a). The effects of ambient air exposures, if any, were attributed to O<sub>3</sub> and any influence of very low (0.04 to 0.07 ppm) concentrations of NO<sub>2</sub> present in the ambient air were unknown.

Several controlled exposure studies used pollutant mixtures containing NO<sub>2</sub> in concentrations from 0.16 to 5.0 ppm. (Kagawa, 1983; Kagawa, 1986; Kleinman et al., 1985; Linn et al., 1986; Stacy et al., 1983; Drechsler-Parks et al., 1987). At concentrations less than 1.0 ppm, these studies demonstrated no obvious effects of NO<sub>2</sub> in pollutant mixtures, which contained O<sub>3</sub>, SO<sub>2</sub>, and/or particles in addition to NO<sub>2</sub>.

Several studies examining the effects of NO<sub>2</sub> concentrations in the range of 0.1 to 0.5 ppm on spirometry in asthmatic subjects suggest possible small changes in lung function (Bauer et al., 1986b; Roger et al., 1985; Koenig et al., 1987 a,b; Avol et al., 1986). However, these changes were absent at higher NO<sub>2</sub> concentrations (Avol et al., 1986; Bylin et al., 1985; Linn et al., 1985b, 1986), thus failing to suggest a concentration-response relationship. Studies examining patients with COPD indicated pulmonary function changes with brief exposure to high concentrations (5 to 8 ppm for 5 minutes) or with more prolonged exposure to lower concentrations (0.3 ppm for 3.75 hours).

A change in airway responsiveness appears to be very sensitive indicator of an adverse response to NO<sub>2</sub> exposure. It has been altered in healthy subjects after exposure to 1.0 ppm or more of NO<sub>2</sub>. Analysis of studies of NO<sub>2</sub> exposure at levels of 0.2 to 0.3 ppm

among asthmatics indicated increased airway responsiveness occurred in some cases from the various challenging agents. These responses appear to be influenced more by the protocol, particularly when subjects exercise during exposure. Several studies examine the effects of exposure to  $\text{NO}_2$  or  $\text{HNO}_3$  exposure on human pulmonary host defense responses. These studies examine the roles  $\text{NO}_2$  exposure may play in potentiating susceptibility to respiratory infections.

### **Effects Of Nitrogen Oxides on Healthy Subjects**

Early studies indicate an effect of  $\text{NO}_2$  exposure on airway resistance at concentrations above 1.5 ppm in healthy volunteers (Beil and Ulmer, 1976; Von Nieding, 1977; 1979). Other studies failed to observe any significant differences in lung function effects from  $\text{NO}_2$  in healthy subjects at concentrations below 1.0 ppm (Folinsbee et al., 1978; Hackney et al., 1978; Beil and Ulmer, 1976; Kerr et al., 1979). This section presents a discussion on these and other studies, as well as studies on NO and  $\text{NO}_2$  mixtures.

### **Lung Function, Respiratory Symptom and Sensory Effects of Nitrogen Dioxide Exposure**

Several studies reported in the other sections also examined symptomatic responses of subjects exposed to  $\text{NO}_2$ . None of the studies of  $\text{NO}_2$  exposure in healthy subjects, including exposure for as long as 75 minutes to 4.0 ppm  $\text{NO}_2$  resulted in a significant increase in respiratory symptoms. Sensory effects were examined in at least two studies (Bylin et al., 1985; Hazucha et al., 1983). The subjects in the study of Hazucha et al. (1983) were unable to detect the odor of 0.1 ppm  $\text{NO}_2$ . Bylin et al. (1985) reported an odor threshold of 0.04 ppm for healthy subjects and 0.08 ppm for asthmatics.

### **Effects at Concentrations Above 1.0 ppm**

The effects of  $\text{NO}_2$  levels greater than 1.0 ppm have been examined in several laboratories. In two studies, von Nieding (1977;1979) studied 11 males exposed to 5.0 ppm  $\text{NO}_2$  for 2 h while performing light, intermittent exercise. Airway resistance increased from 1.51 to 2.41 cm  $\text{H}_2\text{O}$  per L/s after 2 h of exposure. There was also an apparent decrease of arterial oxygen partial pressure ( $\text{PaO}_2$ ) from 90 to 82 torr. These blood samples were taken from "venous" blood drawn from the ear lobe. The statistical analysis of this data is slightly

impacted by an adjustment in the  $\text{PaO}_2$  data prior to testing for significance. Von Nieding and Wagner (1977) have stated: "To increase the power of the tests  $\text{PaO}_2$ -differences <5 mm Hg and RT-increases <0.5 cm  $\text{H}_2\text{O}/\text{L/s}$  were regarded as zero." This transformation increases the likelihood of finding a significant effect. However, pseudo constructs increase the uncertainty in any conclusions drawn from these alterations.

Beil and Ulmer (1976) studied the effects of 2-hour exposures to 0.0, 1.0 (n = 8), 2.5 (n = 8), 5.0, and 7.5 ppm  $\text{NO}_2$  in 16 healthy resting subjects. An additional group of 8 healthy resting subjects were exposed for 14 hour to 5.0 ppm  $\text{NO}_2$  for 2 consecutive days. They found a small significant increase in total respiratory resistance (RT) after exposure to 2.5 ppm  $\text{NO}_2$  or greater. The main response, no more than 1 cm  $\text{H}_2\text{O}/\text{L/s}$  above a baseline of 2.6 cm  $\text{H}_2\text{O}/\text{L/s}$ , occurred during the first 30 minutes of exposure and the response was not appreciably increased by raising the  $\text{NO}_2$  concentration to 5.0 or 7.5 ppm  $\text{NO}_2$ . The increase in RT following  $\text{NO}_2$  exposure was related to the baseline airway responsiveness to acetylcholine. Airway responsiveness to acetylcholine was increased after exposure to 7.5 ppm for 2 h or to 5.0 ppm for 14 hour, but not after the 2-hour exposures to 5.0 ppm or less. The pattern of response in the 14-hour exposure indicated an initial increase in resistance during the first 30 minutes (z 30%), a slight decline in resistance over the subsequent 90 minutes, and then a modest further increase over the next 14 hour (a total increase of =60%). Resistance returned to baseline during the subsequent 10 hour and this response pattern was repeated on the second exposure day. The 1982 Air Quality Criteria for Nitrogen Oxides Document cited this study as indicating that responses were "clearly demonstrated to occur in healthy adults with single two hour exposures to  $\text{NO}_2$  ranging from 4.700 to 14.000 mg/m<sup>3</sup> (2.5 to 7.5 ppm)."

Linn et al. (1985b) exposed 25 healthy, nonsmoking subjects (9 female, 16 male) for 75 minutes to 4.0 ppm of  $\text{NO}_2$  or purified air. Subjects were exposed to each condition twice, for a total of four exposures. The authors reported that approximately 11 mg/m<sup>3</sup> of particulate nitrate was present during  $\text{NO}_2$  exposures. During the exposures, subjects performed 15 minutes of light (25 L/minutes) and 15 minutes of heavy (50 L/minutes) exercise. There were no significant effects of  $\text{NO}_2$  on SRaw or symptoms. Although heart rate and skin conductance were similarly unaffected, there was a slight but statistically significant reduction in systolic blood pressure associated with  $\text{NO}_2$  exposure. Although inhalation of  $\text{NO}_2$  can result in increased blood levels of nitrite and nitrate ion, the

mechanism for this small change in systolic pressure has not been established. Blood pressure readings were obtained using an automated procedure while the subject was seated quietly in the body plethysmograph.

Mohsenin (1987b) studied the effects of one hour resting exposure to 2.0 ppm NO<sub>2</sub> on 11 healthy subjects to determine the effect of ascorbic acid administration prior to NO<sub>2</sub> exposure. The author hypothesized that the antioxidant properties of ascorbic acid would modify the effect of NO<sub>2</sub>. There were a total of four exposures. In the first set of clean air and NO<sub>2</sub> exposures, the subjects received a placebo for 3 days prior to the exposures. In the second air/NO<sub>2</sub> exposure pair, the subjects received vitamin C. In both cases, the order of the NO<sub>2</sub> and air exposures were randomized and blood ascorbic acid levels measured. The levels were increased from 0.76 mg/dl after placebo to 1.90 mg/dl after vitamin C supplementation. Neither plethysmography nor spirometry tests indicated a significant effect of NO<sub>2</sub> in these subjects under placebo or vitamin C conditions. There was a significant increase in airway responsiveness to methacholine (bromide) after NO<sub>2</sub> exposure. Response to methacholine was quantified by the dose required to reduce SGaw by 40% (PD40); this corresponds to a 67% increase in SRaw. (A 50% decrease in SGaw corresponds to a doubling of SRaw.) After the two air exposures, PD40 averaged about 64 mg/ml, but was reduced to 53 mg/ml after NO<sub>2</sub> exposure and placebo treatment. When the subjects were given ascorbic acid prior to exposure, methacholine responsiveness after NO<sub>2</sub> exposure was unchanged. Ascorbic acid pretreatment seemed to block the increase in airway responsiveness previously observed with NO<sub>2</sub> exposure, although it had no effect on baseline methacholine responsiveness. However, ascorbic acid has previously been shown to cause a decrease in methacholine responsiveness in both healthy and asthmatic subjects (Mohsenin et al., 1983; Ogilvy et al., 1981). Thus, it is unclear whether ascorbic acid blocks the effect of NO<sub>2</sub> on airways responsiveness or whether there was a direct effect of ascorbate on methacholine responsiveness subsequent to vitamin C supplementation.

Mohsenin (1988) studied the response of 18 healthy adults exposed to 2 ppm NO<sub>2</sub> for 1 h at rest. There were no symptoms, no changes in lung volume, no change in flow-volume characteristics on either full or partial expiratory flow-volume (PEFV) curves, and no change in SGaw. However, airway responsiveness to methacholine was increased following exposure in 13 of 18 subjects and decreased in only 2 of the 18 ( $p = 0.003$ ) subjects. The

dose of methacholine needed to cause a 40% reduction in SGaw was  $101 \pm 44$  mg/ml after air and  $81 \pm 45$  mg/ml after  $\text{NO}_2$ .

Kulle and Clements (1988) studied the effects of  $\text{NO}_2$  exposure on infectivity of live attenuated influenza A/Korea virus in healthy nonsmoking adults (see Goings et al., 1989). Independent control and exposure groups were exposed to clean air for one day and then either clean air or  $\text{NO}_2$  (1, 2, or 3 ppm) for the next three consecutive days. Included in this evaluation were measurements of respiratory symptoms, lung function, and airway reactivity to methacholine in the 2-ppm and 3-ppm studies. There were no significant changes in respiratory or other symptoms as a result of a 3-ppm  $\text{NO}_2$  exposure. The only apparently significant changes in spirometry were observed in the control group, who showed slightly less decrease in forced vital capacity (FVC) or forced expiratory flow at 25 to 75 % of vital capacity (VC) (FEF2s 75%) during the last of four consecutive clean air exposures. Airway responsiveness to methacholine was measured following exposure to 2 ppm and 3 ppm. The clean air control groups showed a small significant decrease in airway responsiveness on the second, third, and fourth days, but airway responsiveness remained unchanged in the  $\text{NO}_2$ -exposed subjects. Influenza virus infection did not alter airway responsiveness in either air or  $\text{NO}_2$  exposure groups. Reactivity returned to control at two and four weeks after the exposure.

Frampton et al. (1991) studied a group of 39 healthy nonsmokers exposed for three hours to either 0.60 ppm ( $n = 9$ ), 1.5 ppm ( $n = 15$ ), or under a variable concentration protocol, where three 15 minute "peaks" of 2.0 ppm were added to a background level of 0.05 ppm ( $n=9$ ). There were no direct effects on pulmonary ventilatory function (FVC, FEV1, SGaw) after these exposures. There was a small statistically significant increase in FVC and FEV1 response after carbachol challenge in the group exposed to 1.5 ppm, suggesting an increase in airway responsiveness. There was no increase in airway responsiveness among those subjected to continuous exposure of 0.6 ppm exposure or from the 0.5 plus three 15 min. peaks of 2.0 ppm protocol. The higher concentration extended duration exposure did evoke a response, however, the background plus peaks exposures failed to elicit any response. One subject had a 20% greater drop in FEV<sub>1</sub> after the peak  $\text{NO}_2$  exposure than after air exposure, indicating some subjects appear to be more sensitive to  $\text{NO}_2$  than others. This study also suggests that a low level TWA with intermittent high peaks does not appear to be harmful.

### **Concentrations Below 1.0 ppm**

In NO<sub>2</sub> exposure studies conducted at concentrations below 1.0 ppm, the findings have been generally negative. Although some authors have indicated occasional findings, there does not appear to be a consistent pattern of response at these low NO<sub>2</sub> concentrations that would be indicative of short-term health effects.

Kagawa and Tsuru (1979) studied the effects of 2 h exposure of 0.15 ppm NO<sub>2</sub> on six healthy men while performing light, intermittent exercise. There were no symptoms reported during NO<sub>2</sub> exposure. Although the authors suggested that there might be some responses to NO<sub>2</sub> exposure, the overall pattern of response does not support the conclusion that changes in lung function were induced by NO<sub>2</sub>. These authors reported "significance" for individual subjects, although the precise technique for making this judgment is unclear. Two mean differences were reportedly "significant" (multiple t-test unadjusted for multiple comparisons), due to a 0.5% decrease in VC and a 16% decrease in FEF. However, non-significant responses of greater magnitude were observed under other exposure conditions (e.g., air control). It appears that these "significant" observations may only be chance occurrences out of nearly 100 t-tests, 6 of which showed "significance." Furthermore, a temporary 0.5% decrease in VC is of little, if any, physiological significance.

Subsequently, Kagawa (1983) reported the results of exposing an seven additional subjects to 0.15 ppm NO<sub>2</sub> (and to other pollutants, separately or in combination) for two hours with light, intermittent exercise. Using the same protocol and exposure conditions as those of Kagawa and Tsuru (1979), no statistically significant changes were found associated with NO<sub>2</sub> exposure in any of the plethysmograph or spirometric tests. Toyama and colleagues (1981) exposed five healthy subjects (two were smokers; three were investigators) to 0.7 ppm NO<sub>2</sub> for 60 minutes while at rest. They observed no responses to this NO<sub>2</sub> exposure that altered airway conductance or flow-volume tests.

Kerr et al (1979) reported a study of 10 normal, 13 asthmatic, and 7 chronic bronchitis subjects who were exposed to 0.5 ppm NO<sub>2</sub> for two hours; several of the subjects were smokers (3 normal, 3 asthmatics, and 5 bronchitis). There were no significant effects of NO<sub>2</sub> exposure on pulmonary function; a change in quasistatic compliance was observed but it was unclear whether this was due to NO<sub>2</sub> exposure or was a statistical artifact, as the authors suggested.

Rather than compare data across the post-exposure measurements of clean air and NO<sub>2</sub> exposures respectively, a reanalysis of the difference in scores (pre vs. post) was determined for each condition and the differences were tested for significance. All subjects indicated they perceived an odor of NO<sub>2</sub> upon entering the exposure chamber. In the healthy subjects, the authors reported a significant increase in the Phase IV single breath nitrogen washout test. However, upon analysis, they suggested the difference was due to a lower pre-exposure value on the NO<sub>2</sub> exposure day. This effect could not be attributed to NO<sub>2</sub>. The semi static lung compliance was decreased after NO<sub>2</sub> exposure in the healthy group. The absence of a change in dynamic compliance suggests that the original authors (Kerr et al., 1979) may have been correct in concluding that "significance" was probably due to chance alone. No other pulmonary measurements were significantly altered by NO<sub>2</sub> exposure. With the exception of the apparently artifactual change in closing volume, no new conclusions can be drawn from the reanalysis of these data.

Stacy et al. (1983), as part of a large multipollutant exposure study, exposed a group of 10 men to 0.5 ppm NO<sub>2</sub> for four hours, including two 15-minutes periods of moderately heavy exercise. None of the plethysmographic or spirometric tests showed any significant effects of NO<sub>2</sub> exposure. The experimental design of this study was complex, having a total of 20 treatment cells. The data were statistically analyzed by multivariate analysis of variance with an adjusted p value and by individual t-tests with a p value of 0.05. Neither analysis indicated significant effects of NO<sub>2</sub>.

Hazucha et al. (1982, 1983) studied a group of 15 healthy adult males exposed to either air or 0.1 ppm NO<sub>2</sub> for one hour. Control measurements were performed on the day before and the day after the exposure. The subjects did not detect the odor of NO<sub>2</sub>; nor was there an increase of symptoms related to the NO<sub>2</sub> exposure. There were no effects of NO<sub>2</sub> on spirometry, airway resistance (SRaw or RT)~ or methacholine responsiveness.

Rehn et al. (1982) reported a small (17%) increase in SRaw among eight healthy men after exposure to 0.27 ppm (0.500 mg/m<sup>3</sup>) for one hour. No response was seen at 1.06 ppm (2.0 mg/m<sup>3</sup>).

Bylin et al. (1985) exposed eight healthy subjects to 0.230, 0.460, and 0.910 mg/m<sup>3</sup> (0.12, 0.24, and 0.48 ppm) for 20 minutes. An analysis of variance did not reveal any significant effects of NO<sub>2</sub> on changes in SRaw, but specific comparisons indicated a significant 11 % increase in SRaw at 0.24 ppm and a 9% decrease in SRaw at 0.48 ppm.

Even though statistically significant, such small changes (+15%) in airway resistance are well within the normal variation of 10 to 20%. Histamine bronchial responsiveness was tested after the 0.48-ppm exposure, but there were no changes.

Koenig et al. (1987a) exposed healthy subjects to (1) 0.12 ppm NO<sub>2</sub> for two 30-minutes periods at rest, or (2) to 0.12 ppm for 30 minutes at rest plus 10 minutes with exercise, or (3) to 0.18 ppm for 30 minutes at rest and 10 minutes of exercise. For the at-rest 0.12-ppm NO<sub>2</sub> exposures, there were no significant changes in lung function, symptomology, or arterial oxygen saturation. Nor were there significant NO<sub>2</sub> effects on lung function with the mild exercise groups exposed to 0.12 and 0.18 ppm NO<sub>2</sub>.

Morrow and Utell (1989) studied both young (20 to 48) healthy subjects and elderly (49 to 69 years old) healthy subjects exposed to 0.3 ppm NO<sub>2</sub> for 3.75 hour. The young subjects performed a total of 30 minutes of moderate exercise during exposure and the older subjects exercised for 21 minutes. There were no differences between air exposure and NO<sub>2</sub> exposure for symptom responses, changes in lung function, or in airway responsiveness to carbachol in either young or older subjects.

The effects of 0.60-ppm NO<sub>2</sub> exposures on young men and women during one hour of continuous heavy exercise were studied by Adams et al. (1987). There were no significant effects of NO<sub>2</sub> exposure on airway resistance, symptoms, spirometry, or exercise responses.

Kim et al. (1991) studied nine athletes exposed to filtered air, or to 0.18, and 0.30 ppm NO<sub>2</sub> for 30 minutes while exercising. Sixteen minutes were spent running at a ventilation rate of 72 L/minutes; 10 of the remaining 14 minutes were spent walking. Overall ventilation is estimated to have averaged about 50 L/minutes. There were no significant changes in respiratory symptoms, FEV1, RT, peak expiratory flow rate, or ventilation (V50%VC) as a result of NO<sub>2</sub> exposure in this group of athletic male subjects.

In another study, young (18 to 26 years old) and older (51 to 76 years old) men and women performed light, intermittent exercise during 2 h exposures to 0.60 ppm NO<sub>2</sub> (Drechsler-Parks et al., 1987; Drechsler-Parks, 1987). Subjects were tested and no effects were detected on spirometry nor were there symptoms of ill effects. None of the individual pre-post exposure differences in NO<sub>2</sub> (as compared to air) for FVC or FEV1 were outside of the normal range (no individual subjects appeared reactive to NO<sub>2</sub>).

### **Mucociliary Clearance After Nitrogen Dioxide Exposure**

Rehn et al. (1982) examined the effects of NO<sub>2</sub> exposure on mucociliary clearance in both the nose and lung. Nasal clearance was determined using the rate of saccharin transport from nares to oropharynx. Tracheobronchial clearance was determined by monitoring the rate of disappearance of radiolabeled Teflon aerosol. After a one hour exposure to either 0.27 or 1.06 ppm (500 or 2,000 yg/m<sup>3</sup>) NO<sub>2</sub>, there were no changes in either nasal or tracheobronchial clearance rates.

### **Summary of Effects of NO<sub>2</sub> on Healthy Humans**

In the studies summarized above, observations of increases in SRaw were reported. At concentrations in excess of 2.0 ppm, functional changes occurred in the lungs of healthy volunteers that might be attributed to NO<sub>2</sub> exposure. However, this is contradictory to other studies where, normal health humans are unaffected by 2.0 ppm (Devlin, 1992), or by 2.25 ppm (Sandstroem (1989), or in asthmatics who were unaffected by 4.0 ppm (Linn et al, 1985b). Exposure to NO<sub>2</sub> may increase airway responsiveness that has been established by other substances. It also raises the possibility that NO<sub>2</sub> could influence pulmonary disease and a major concern would be with COPD patients, due to a lack of satisfactory pulmonary reserves.

### **Effects Of Nitrogen Dioxide On Asthmatics**

An important issue in the evaluation of human clinical exposure studies involving asthmatics is the variability in response between, and within analytical laboratories. In the absence of significant differences in the exposure protocol or exposure dose, an explanation frequently presented to explain differences in responsiveness is that the severity of the disease can differ from one subject group to another. An Expert Panel from the National Asthma Education Program of the National Heart Lung and Blood Institute, National Institutes of Health, defines asthma as a pulmonary disease with the following characteristics: (1) airway obstruction that is reversible (but not completely so in some patients) either spontaneously or with treatment; (2) airway inflammation; and (3) increased airway responsiveness to a variety of stimuli.

**Table 4.1**  
**SUMMARY OF STUDIES OF HEALTHY SUBJECTS EXPOSED TO NO<sub>2</sub>.**

NO <sub>2</sub> CONC.	EXP. DURATION	NUMBER TESTED	EFFECTS OF EXPOSURE	REFERENCE
4.0-5.0ppm	--	--	Spirometry normal, airway resistance increased	Beil and Ulmer, 1976
0.6ppm	60 min	20 m/20F	No fx on spirometry or airway resistance	Adams et al., 1987
1.0-7.5ppm	120 min	16	increased airway response after 120 min at 7.5 ppm.	Beil and Ulmer, 1976
0.6 ppm	120 min/d x 4d	4M/1F	No fx of repeated exposure on pulmonary function. sl ↑ of polys.	Boushey et al., 1988.
0.12- 0.5ppm	20 min.	5M/4F	Poss ↑ in SRaw @ 0.25ppm	Bylin et al., 1985.
2.0 ppm	240 min	10 normal	↑ polys, ↓AMθ	Devlin et al., 1992
0.60 ppm	120	8M/8F	no sig change in lung function from NO <sub>2</sub>	Drechsler Parks, 1987
0.6 ppm	180 min	7M/2F	No change in spirometry, or SRaw.	Frampton et al., 1989
1.0 ppm	120min/ d x 2 d	16	1.5% ↓ (?) in FVC post 2 d.	Hackney et al., 1978
1.0ppm	180	3M5F	N response observed	Jorres et al. 1992
0.30 ppm	120 min	6	No fx on SRaw. Fx of NO <sub>2</sub> ?	Kagawa, 1986
0.18 ppm	30 min	9M	No change in lung function	Kim et al., 1991
0.12	60 min	4M6F	No fx on lung function	Koenig 1985
0.12	40 min	3M/7F	No fx on R <sub>T</sub> or spirometry	Koenig et al., 1987
0.18	40 min.	4M/6F		
2.0 ppm	60 min	8M/3F	Vit C blocks NO <sub>2</sub> SRaw.	Mohsenin, 1987.
0.27	60 min	Males	Poss sm ↑ SRaw.	Rehn et al., 1982
2.25	20 min	8 Subj.	Mast cells ↑ @ all conc. Lymphs ↑ at 4.0/.5 ppm	Sandstroem et al. 1989.

Fx = effects

↑ = increase

↓ = decrease

SRaw = specific airway response

FVC = Fixed Vital Capacity spirometry

sl = slight

Subj. = subjects

Polys = polymorphonuclear leukocytes

According to the National Institutes of Health, about 10 million people, or 4% of the US population has asthma, and the prevalence is higher among African Americans, older children (8 to 11 years old ), and urban residents (Schwartz et al., 1990). Asthma is typically associated with airway inflammation and epithelial injury and there is a broad range in the degree of severity of asthma, ranging from mild to very severe (Cotran et al., 1996). Common symptoms of asthma include cough, wheezing, shortness of breath, chest tightness, and sputum production. A positive skin test is a response to sensitivity of common allergens and is a typical feature of asthma. The respiratory effects of asthma is characterized by an exaggerated bronchoconstrictor response to many physical changes (e.g., cold or dry air, exercise), chemical and pharmacological agents (e.g., histamine or methacholine).

In addition to basic anthropometric information such as age, height, weight, gender, and race, other information may be useful in characterizing an asthmatic. In order to evaluate differences between subject populations from one study to another, useful information includes baseline lung function, frequency of asthma episodes, nonspecific bronchial responsiveness, reversibility of bronchoconstriction, types of medication and use, specific serum immunoglobulin E (IgE) levels, antigen skin test responses, response to exercise challenge, duration of disease, and the various factors that may precipitate or aggravate symptoms of the disease. Most asthmatic chamber exposures are accompanied by moderate exercise. The potential for an increase in airway resistance or decline in lung volumes or forced expiratory flow caused by exercise alone is a very important covariant in these studies. Exercise, even if moderate, can induce some degree of increase in airway resistance, even in clean air at room temperature and relative humidity (RH), at 20 °C, 50 % RH. In order to determine the true effect of an air pollutant in exercising asthmatics, the response to exercise must be considered. Accordingly, in studies reviewed in this section, a control exposure to clean air was performed, including exercise when appropriate, and calculated ventilation volumes.

Asthmatics who participate in controlled human exposure studies typically have mild allergic asthma. In most cases, such individuals go without medication or may discontinue medication for brief periods of time if exposures are conducted outside the allergic season. Controlled human exposure studies that reported respiratory effects of NO<sub>2</sub> exposure in asthmatics are summarized below.

Symptomatic effects were observed in asthmatics exposed to 0.5 ppm for two hours in a study reported by Kerr et al. (1979). However, only four of the subjects reported symptoms of respiratory discomfort, and the authors concluded that: "The symptoms reported were minimal, did not correlate with functional changes, and are of doubtful significance."

Avol et al. (1988) studied a group of moderate-to-severe asthmatics exposed to clean air, 0.3 ppm, and 0.6 ppm NO<sub>2</sub> for two hours while performing moderate (minute ventilation [VE] = 41 L/min), or intermittent (6 x 10 min) exercise. Each subject was exposed once each to clean air, 0.30 ppm, or to 0.6 ppm. There were significant changes in SRaw and FEV<sub>1</sub> as a function of exposure duration for all exposure conditions, but there was no significant effect of NO<sub>2</sub> exposure on these measures of pulmonary function. Cold air bronchial reactivity (assessed by decrease in FEV<sub>1</sub> after breathing cold-dry air) was measured one hour post-exposure and then again the following day. There was a significant action occurring between the testing and response time (i.e., one hrs post-exposure and 24 hrs post-exposure), suggesting an increased response after exposure to 0.30 ppm, but not after 0.6 ppm. There were no respiratory symptom responses attributable to NO<sub>2</sub> exposure. A post hoc analysis of a subgroup of subjects with the most abnormal lung function (i.e., FEV<sub>1</sub>/FVC ratios < 0.65) revealed no statistically significant effects of NO<sub>2</sub>. In addition to the controlled exposures, 36 subjects also were exposed to ambient air containing 0.09 ppm NO<sub>2</sub> and low levels of other pollutants. Neither lung function, cold-air reactivity, nor symptom responses were significantly different in ambient air than in clean air.

Bauer et al. (1986a) reported a statistically significant spirometric response to NO<sub>2</sub> in a group of 18 asthmatics exposed to 0.3 ppm NO<sub>2</sub> by mouthpiece for 20 minutes at rest followed by 10 minutes of exercise (30 L/min). These subjects were characterized as having "mild obstructive lung disease (asthma)." All subjects had elevated response to cold air bronchoprovocation. Nitrogen dioxide deposition studies indicated that 72 % (at rest) and 87 % (during exercise) of the inhaled NO<sub>2</sub> was deposited within the respiratory tract. According to the authors, the measurements of NO<sub>2</sub> deposition were in general agreement with the model predictions of Miller et al. (1982). After NO<sub>2</sub> exposure, 9 of 15 asthmatics had a decrease in FEV<sub>1</sub> relative to their post exercise FEV<sub>1</sub> in clean air. The post exercise FEV<sub>1</sub> was 4.1 % lower after NO<sub>2</sub> than after air exposure; the pre- to post exposure

difference on the NO<sub>2</sub> day (10.1 %) and the pre- to post NO<sub>2</sub> minus the pre- to post-air (i.e., delta-delta) differences (6%) were significant using a paired t-test. These differences were no longer present by 60 minutes after the exposure. Maximum expiratory flow at 60% total lung capacity (TLC) (PEFV curve) was decreased more after NO<sub>2</sub> exposure than after air exposure. Changes in FVC and SGaw appear to be unaffected between air and NO<sub>2</sub> exposures. Airway responsiveness to cold air in this study was determined also. At each ventilation rate of cold air breathing, the respiratory heat exchange (RHE) was calculated [RHE, is a relationship of the log RHE versus the percentage decrease in FEV<sub>1</sub> causing a 10% decrease in FEV<sub>1</sub>], and was linearly interpolated and referred to as a provocative dose in RHE units needed to decrease FEV<sub>1</sub> by 10%. Of the 12 subjects for whom this dose could be determined, 9 showed an increased response to cold air after the NO<sub>2</sub> exposure. The average provocative RHE dose decreased from 0.83 kcal/min after air exposure to 0.54 kcal/min after NO<sub>2</sub> exposure. One factor that may have led to the increased response after exposure to a low concentration of NO<sub>2</sub> in this group of asthmatics could be the fact that a mouthpiece exposure system containing relatively dry air (RH of 9 to 14% at 20 °C) was used, and that there was possibly some interaction between the NO<sub>2</sub> effect and airway drying. It has been established that breathing dry (cold) air induces bronchoconstriction in asthmatics; the effects of sulfur dioxide exposure, for example, has a heightened effect on asthmatics and the asthma is worsened by oral inhalation of cold-dry air (U.S. Environmental Protection Agency, 1992). Concern over this confounding effect is tempered by the fact that Bauer et al. (1986) controlled for the airway drying effect by exposing subjects to clean air at the same temperature and RH. However, if the formation of HNO<sub>3</sub> or nitrous acid is potentially involved in the observed responses, the air chemistry could be strongly influenced by RH. Sequestration of HNO<sub>3</sub> on surfaces is increased with increased ambient water vapor content.

Eight asthmatics exposed to 0.0ppm, 0.1ppm, 0.25ppm, and 0.5 ppm NO<sub>2</sub> for 20 minutes were studied by Bylin et al. (1985). Exposures were conducted in a body plethysmograph and the range of concentrations was +18% to -26% of the target concentration. Changes in SRaw during the four exposures averaged +3%, +9%, -2%, and -14%, respectively. Three-way analysis of variance revealed no significant differences in SRaw due to NO<sub>2</sub> exposure. There was a tendency for the pre to post exposure difference for thoracic gas volume (TGV) to be larger for the NO<sub>2</sub> exposures (9 to 10%). However, the

absolute volume of TGV was at most 3 to 4% lower than at comparable times in other NO<sub>2</sub> exposures and only 2 % less than the air exposure. The significance of the difference was a higher pre exposure value of the 0.1-ppm and 0.5-ppm NO<sub>2</sub> exposures; this effect, if real, is not attributable to NO<sub>2</sub>.

There were no significant changes in tidal volume or respiratory rate, which would have been suggestive of an irritant response. At the highest NO<sub>2</sub> concentration tested (0.5 ppm), histamine induced bronchial responsiveness was evaluated after exposure. The authors report a significant increase in histamine initiated responsiveness due to NO<sub>2</sub> exposure. Significance was evaluated by a sign test ( $p < 0.04$ ; responsiveness increased in five subjects and was unchanged in three). However, this finding should be interpreted cautiously because the air exposure histamine challenge had to be discontinued in two subjects, one of whom was later classified as having increased responsiveness. Five of the eight asthmatics had previously been hyperactive to histamine but were not at the time of the NO<sub>2</sub> exposures. This paper suggested possible increased histamine reactivity after 0.50 ppm NO<sub>2</sub> exposure of asthmatics but no direct effect of NO<sub>2</sub> on SRaw at concentrations up to 0.5 ppm for 20 minutes. The increased responsiveness and later lack of responsiveness is somewhat contradictory and precludes any supportive conclusions from the data developed among this group.

Bylin et al. (1988) reports 0.26, 0.51, and 1.0 mg/m<sup>3</sup> (0.14, 0.27, 0.53 ppm NO<sub>2</sub> respectively) exposure on a group of 20 mild asthmatics failed to produce any significant changes in SRaw, although there was a general trend for SRaw to fall throughout the period of exposure regardless of the pollutant level. The challenge and stress of testing appears to have played a role in altering responsiveness since there was, a significant increase ( $p = 0.03$ ) in airway responsiveness to histamine after 30-minutes exposure to the middle concentration (i.e., 0.510 mg/m<sup>3</sup>), but no response developed at the lowest and highest concentrations. The lack of a dose related increase in responsiveness, and the fact that these findings are based on repeated application of a non parametric test without an adjusted alpha level ( $p$  value) for multiple comparisons, suggests these results may be flawed. This observation contrasts with an earlier observation (Bylin et al., 1985) that suggests possible increased responsiveness after exposure to 0.91 mg/m<sup>3</sup>. The raw data presented in the paper was subjected to reanalysis using a Friedman non-parametric analogue of an F test, which is considered more appropriate for these data than the series

of Wilcoxon matched signed pairs rank test. The Friedman test showed no difference across treatment groups and there was no statistically significant increase in histamine responsiveness as a result of NO<sub>2</sub> exposure.

In a study that was an important precedent to a number of studies, Orehek et al. (1976) studied the effects of low levels of NO<sub>2</sub> exposure on the bronchial sensitivity of mild asthmatic patients to carbachol, a bronchoconstricting agent. Exposures took place in an airtight room. Nitrogen dioxide concentration started at 0.246 mg/m<sup>3</sup> (0.13 ppm) and declined to 169 µg/m<sup>3</sup> (0.09 ppm) over 60 minutes; the average concentration was 210 µg/m<sup>3</sup> (0.11 ppm). Changes in SRaw from pre- to post exposure were measured and an airway challenge to carbachol was used to assess post exposure airway responsiveness. Following NO<sub>2</sub> exposure, increases in SRaw were observed in only 3 of 20 asthmatic test subjects. For all 20 of the asthmatic subjects, dose-response curves were developed for changes in SRaw as a result of subjects inhaling carbachol. These response curves were compared after a 1-hrs exposure to either clean air or NO<sub>2</sub>. Nitrogen dioxide exposure was associated with increased airway responsiveness to carbachol in 13 of 20 subjects. The mean dose of carbachol producing a 100% increase in SRaw in the 13 most sensitive responders exposed to NO<sub>2</sub> was significantly decreased from 0.66 mg to 0.36 mg. Seven of the asthmatic subjects (non responders) showed neither an increase in SRaw in response to the exposure to NO<sub>2</sub> alone nor an enhanced effect of NO<sub>2</sub> on carbachol-induced bronchoconstriction. The results of this study are suggestive that certain individuals are specifically sensitive, and very low concentrations of NO<sub>2</sub> will produce responses in some asthmatics. A criticism of this report is that comparisons of SRaw were made in subjects who were selected, not at the time of NO<sub>2</sub> exposure, but after the fact, following the carbachol challenge. For example, the mean of measurements of SRaw in the 13 responders to the carbachol treatment was significantly higher after the NO<sub>2</sub> exposure than it had been prior to exposure. A criticism of this study discussed by Hazucha et al., (1983) is that, "in addition to the retrospective stratification of subjects into responders and non-responders, other statistical methods may have been more appropriate than the selected group of paired t-tests used in this study".

Orehek et al. (1981) also studied seven allergic subjects, three of whom had asthma, who were exposed to 0.11 ppm NO<sub>2</sub> for one hour. The major hypothesis to be tested was that NO<sub>2</sub> may alter bronchial responsiveness to an inhaled allergen. Studies were

conducted outside the typical pollen season. The exposure technique was somewhat primitive since  $\text{NO}_2$  was added to the exposure room with a starting concentration of 0.16 ppm, which was allowed to decay during the exposure to a concentration of 0.07 ppm. There was no change in SRaw or symptoms as a result of  $\text{NO}_2$  exposure. There was no difference in SRaw response to allergen challenge in these subjects. [ $\text{NO}_2$  did not act synergistically with allergen challenge.] There was no difference in responses of the three asthmatic and the other four subjects.

Hazucha et al. (1982, 1983) published two reports that contain complementary data from a study in which the Orehek protocol was repeated. In contrast to the report of Orehek et al. (1976), Hazucha et al. (1982, 1983) found no statistically significant change in airway reactivity to methacholine in a group of 20 mild asthmatics following a 1 h resting exposure to 0.1 ppm  $\text{NO}_2$ . A small (8%) increase in SRaw ( $p = 0.23$ ) was observed after  $\text{NO}_2$  exposure. Three of the 15 subjects had a greater than 20% decrease in the dose of methacholine required to double SRaw. However, at least three of the subjects had a change of similar magnitude in the opposite direction, based on the graphical presentation of the methacholine dose-response curves. Respiratory system resistance measured by the forced oscillation method was not changed by  $\text{NO}_2$  exposure. Hazucha et al. (1983) suggested that the difference in the conclusions regarding statistical significance reached by Orehek et al. (1976), despite similar findings, was because "the statistical approach used by Orehek was not appropriate." Hazucha et al. (1983) discussed the factors that led to their conclusions and noted had they analyzed their data in a similar manner to Orehek et al. (1976), the findings would have been comparable.

The concept that  $\text{NO}_2$  exposure may cause airway hyperresponsiveness was examined by Kleinman et al. (1983), who employed a different experimental design than Orehek et al. (1976) and Hazucha et al. (1983). They studied 31 mild-to-moderate asthmatics who were exposed to 0.2 ppm  $\text{NO}_2$  for two hours while performing light, intermittent exercise. There were no significant effects of  $\text{NO}_2$  exposure on forced expired spirometry. Total RT (forced oscillation) tended to increase (9%) after  $\text{NO}_2$  exposure, but the difference was not significant ( $p = 0.11$ ). Symptom responses tended to be slightly higher after air exposures. A number of different methods were used to evaluate the methacholine challenge data. The general tendency was for greater responsiveness to methacholine after  $\text{NO}_2$  exposure. The determination of the dose that would cause a 10% decrease in FEV1 (D10) was the

most "conventional" approach (O'Connor et al., 1987, to assessing methacholine responsiveness. In the 21 subjects in which this dose could be ascertained, D10 was  $8.6 \pm 16.2 \mu\text{g}$  on the air day and  $3.0 \pm 6.2 \mu\text{g}$  on the  $\text{NO}_2$  days ( $p < 0.05$  by t-test and Wilcoxon test). It appears that the results of this study suggest a possible increase in airway responsiveness after a two hours exposure to 0.20 ppm  $\text{NO}_2$ .

Koenig et al. (1985) have studied the effects of a one hour resting exposure of asthmatic adolescents to 0.12 ppm  $\text{NO}_2$ . There were no "consistent significant changes in pulmonary functional parameters" after  $\text{NO}_2$  exposure. Although symptom data were not presented, the authors indicated that subjects had more symptoms after  $\text{NO}_2$  exposure but that the trend was not significant.

Subsequent studies by Koenig et al. (1987a,b) of mouthpiece exposures to 0.12 ppm  $\text{NO}_2$ , which incorporated exercise (30-minutes rest followed by 10-minutes exercise), indicated increases in RT and decreases in FEV1 after both air and  $\text{NO}_2$  exposure. These changes were apparently due to exercise alone (RT increased 8.1% with air and 10.4% with  $\text{NO}_2$ ; post exercise FEV1 was decreased 7.4% with air and 4.1 % with  $\text{NO}_2$ ). In the final phase of the study, subjects were exposed to 0.18 ppm  $\text{NO}_2$  using the same exercise protocol. In this case, no differences in RT were seen and FEV1 decreases were 1.3 and 3.3 % for air and  $\text{NO}_2$ , respectively. This difference ( $p = 0.06$ ) may indicate a possible response trend. There were no differences in symptoms between exposure conditions in either the 0.12- or 0.18-ppm  $\text{NO}_2$  exercise exposure studies.

Morrow and Utell (1989) studied a group of 20 asthmatics exposed to 0.30 ppm  $\text{NO}_2$  for 3.75 hours. The exposure included three 10-minutes periods of moderate exercise. There were no statistically significant group changes in symptoms, spirometry, plethysmography, or airway reactivity to carbachol as a result of the  $\text{NO}_2$  exposure. Some of the subjects ( $n = 7$ ) had participated in the Bauer et al. (1986) study. The 13 remaining (new) subjects were judged to have more severe asthma than the "repeaters." Although the repeaters tended to have responses that were similar to those in the previous study (larger FEV1 decrements in  $\text{NO}_2$  than in air), the new subjects had significantly greater FEV1 decrements during the air exposures. Linn et al. (1985) and Linn and Hackney (1984) exposed a group of 23 mild asthmatics to 4 ppm  $\text{NO}_2$ . Subjects completed a total of four exposures, two each to  $\text{NO}_2$  and clean air, separated by one week. Exposures lasted for 75 minutes and included two 15-minutes exercise periods separated by a 25-minutes rest period. The first exercise was

light (25 L/min) and the second was heavy (49 L/min). All subjects were responsive to inhaling 0.75 ppm SO<sub>2</sub> during exercise. Mean baseline pre-exposure SRaw measurements varied from 5.48 and 5.59 on the air exposure days to 6.14 and 6.44 on the NO<sub>2</sub> exposure days, although it is unlikely that the slightly higher baseline values on the NO<sub>2</sub> exposure days affected the subjects' responses. Airway resistance increased after exercise and more so after the heavy (57.2%) than after the light (17.6%) exercise. Percentages represent mean values across all exposure conditions. There was no significant differences in lung function that could be attributed to NO<sub>2</sub>; if anything, SRaw tended to be slightly lower with the NO<sub>2</sub> exposures. Physiological tests, such as skin conductance and heart rate, were not different due to exposure conditions. As with the group of healthy subjects studied under similar conditions, these asthmatics had a slightly, but significantly lower, systolic blood pressure towards the end of the NO<sub>2</sub> exposure. The authors suggested the possibility that NO<sub>2</sub> deposited in the respiratory tract may form a vasoactive substance such as an organic or inorganic nitrate. Cotran et al (1995) describe the presence of endogenously produced nitrous oxide; that may contribute to nitrate formation and subsequent effects on blood pressure. After NO<sub>2</sub> inhalation, nitrate formation has been observed in animal studies (Postlethwait and Mustafa, 1982). Measurements of blood nitrate levels were not performed by Linn et al. (1985). Both symptoms and anxiety scores were evaluated during and after exposure; there were no significant variations that could be attributed to NO<sub>2</sub> exposure. It is difficult to explain the differences between this group of asthmatics exposed to 4 ppm for 75 minutes (with exercise) compared to the group exposed to 0.30 ppm for 30 minutes with exercise studied by Bauer et al. (1986). The subjects of Bauer et al. were exposed to NO<sub>2</sub> in dry air through a mouthpiece, which could have caused some "drying" of the upper airways. This would not be a factor in the Linn et al. (1985) study, where a chamber exposure was used. Another possible explanation is that the asthmatics studied by Linn et al. were accustomed to NO<sub>2</sub> exposure because of their place of residence (Los Angeles ambient levels are generally lower than 4.0 ppm, but could also be higher.). The indoor environment can also be an important avenue of NO<sub>2</sub> exposure, but it is not known for either group. Secondly, the asthmatics in the Linn et al. study, although reactive to SO<sub>2</sub>, tended to have milder disease; none used regular asthma medications and all but three subjects had an FEV1/FVC ratio in excess of 75 % .

All of the subjects in the Bauer et al. study used some form of bronchodilator (oral or inhaled) and 9 of 15 subjects had a baseline FEV<sub>1</sub>/FVC ratio less than 75 %. It is not clear whether the effects of NO<sub>2</sub> could have been confounded by exposure to an ambient airborne allergen. Although subjects in the Linn et al. study were exposed in March, a time when outdoor pollen air allergen tended to be minimal for the several preceding months, the winter is a peak season for fungal aeroallergen (Street and Hamburger, 1976; McLean et al., 1991). Also, increased bronchial reactivity to cold air has been an important finding in the Bauer et al. study; but was not measured in the Linn et al. study.

Further studies were conducted by Linn et al. (1986) on 21 (minimal to mild) asthmatics exposed to 0, 0.30, 1.0, and 3.0 ppm NO<sub>2</sub> for one hour. The exposures included intermittent, moderate exercise (VE = 41 L/min). This group was characterized as "clinically mild extrinsic (allergic)" asthmatics who required infrequent, if any, medication. As in the previous study with 4.0 ppm NO<sub>2</sub> exposures, there were no significant effects of NO<sub>2</sub> on spirometry, SRaw, or symptoms. Furthermore, there was no significant effect on airway reactivity as measured by cold-air challenge. In order to examine the suggestion that the severity of response to NO<sub>2</sub> may be related to the clinical severity of asthma, the authors selected three subjects whom they characterized as having more severe illness. There was no indication that the responses of these subjects were related to NO<sub>2</sub> exposure, although they experienced markedly larger changes in resistance than other milder asthmatics under all exposure conditions. Heart rate or minute ventilation did not vary significantly with NO<sub>2</sub> exposure and the previously observed decrease in systolic pressure, associated with 4.0 ppm NO<sub>2</sub> exposure, was not examined in these subjects.

Mohsenin (1987) studied 10 mild asthmatics exposed to 0.5 ppm NO<sub>2</sub> for one h at rest in an exposure chamber. There were no changes in symptoms, spirometry, or plethysmography that could be attributed to NO<sub>2</sub> exposure. The response to methacholine bromide was evaluated with partial expiratory flow at 40% VC (PEF40%VC), rather than changes in SRaw or FEV<sub>1</sub>, to test for "small airway abnormality" without the influence of prior deep breaths. There was a significant increase in airway responsiveness to methacholine after the NO<sub>2</sub> exposure. The dose of methacholine required to decrease PEF40%VC by 40% was 9.2 ± 15 after air and 4.6 ± 8.2 after NO<sub>2</sub> (p = 0.042).

Roger et al. (1990) reported the results of NO<sub>2</sub> exposure in mild asthmatics. The first was a pilot study of 12 mild asthmatics exposed to 0.30 ppm for 110 minutes, including

three 10-minutes periods of exercise. After the first 10 minutes of exercise in NO<sub>2</sub>, they found an 11% decrease in FEV<sub>1</sub>, which was significantly larger than the 7% decrease seen after the clean air exposure. These differences between air and NO<sub>2</sub> exposure persisted for the remainder of the exposure period, although the overall responses were progressively less with successive periods of exercise, as is common with exercise-induced asthma when the exercise stimulus is intermittent.

A concentration-response study was subsequently conducted (Roger et al., 1990) with 21 mild asthmatics, including 6 subjects from the pilot study, who were exposed to 0.0, 0.15, 0.30, and 0.60 ppm NO<sub>2</sub>. The 75-minutes exposures included three 10-minutes exercise periods. In contrast to the pilot study, there were no differences in response between the air and NO<sub>2</sub> exposure at any exposure concentration or time during the exposure. Bronchial reactivity to methacholine, tested two hours after the exposures, was similar for air and NO<sub>2</sub> exposures. There were no significant differences in symptom scores across the four exposure conditions. The authors were unable to specifically identify factors that could have caused the difference in response between the pilot study and the larger, more comprehensive concentration-response study. They suggested that the pilot study asthmatics may have had more reactive airways, based on their poorer baseline lung function and greater airway responsiveness to methacholine compared to the subjects in the concentration-response study. Furthermore, the studies were conducted during different seasons, which may account for some of the variability in response.

Rasmussen et al. (1990) presented a preliminary report of a concentration-response study of healthy asthmatic subjects exposed to 0.1, 0.2, and 0.8 ppm NO<sub>2</sub>. Exposures lasted 120 minutes and included 10 minutes of exercise. There were no significant changes in lung function (SRaw, FEV<sub>1</sub>) or airway responsiveness to histamine resulting from NO<sub>2</sub> exposure at any concentration in either healthy or asthmatic subjects. Acoustic rhinometry, nasal mucociliary clearance, and alveolar epithelial permeability were also examined, but these results were not reported.

A series of abstracts have been presented by investigators from Mt. Sinai Medical Center in Miami (Sackner et al., 1980; Ahmed et al., 1983); these reports have not appeared in the peer-reviewed literature but are available as technical reports (Ahmed et al., 1983). The latter report presents data that are qualitatively similar to Orehek et al. (1981) and Hazucha et al. (1983) in that some subjects (13 out of 20) showed increased

airways responsiveness to carbachol after  $\text{NO}_2$  exposure and some (7 out of 20) did not. Even with the *post hoc* separation of subjects into "reactive" and "non reactive" groups, the increase in airway responsiveness in the reactive group ( $n = 13$ ) was not statistically significant. There were no significant changes in lung function. Adequate characterization of the exposure conditions was not presented. The former report (Ahmed et al., 1983) dealt with effects of  $\text{NO}_2$  on nine ragweed-sensitive asthmatics. There were no group mean changes in SGaw or FEV1 after  $\text{NO}_2$  exposure. Also, no change in bronchial responsiveness to a ragweed antigen inhalation challenge either immediately or 24 hours after exposure to 0.1 ppm  $\text{NO}_2$  was observed.

The effects of prior  $\text{NO}_2$  exposure using sulfur dioxide induced bronchoconstriction has been examined in two studies. Jorres and Magnussen (1990) exposed 14 mild-to-moderate asthmatic subjects to 0.25 ppm  $\text{NO}_2$  for 30 minutes while breathing through a mouthpiece at rest. There were no changes in SRaw as a result of the exposure. After exposure, airway responsiveness was assessed by isocapnic hyperventilation of 0.75 ppm  $\text{SO}_2$  using stepwise increases in ventilation; the initial level was 15 L/min with subsequent increases to 30, 45, 60 L/min, and so forth. After a 3-minutes period of hyperventilation, SRaw was determined. The ventilation of  $\text{SO}_2$  required to produce a 100% increase in SRaw was estimated by interpolation of ventilation versus SRaw (dose-response) curves. The provocation dose was significantly reduced after  $\text{NO}_2$  exposure compared to that occurring after filtered air exposure, suggesting that the airways were more responsive to  $\text{SO}_2$  as a result of the prior  $\text{NO}_2$  exposure.

Rubinstein et al. (1990) exposed nine asthmatics to 0.30 ppm  $\text{NO}_2$  for 30 minutes (including 20-minutes light exercise). An  $\text{SO}_2$  bronchoprovocation test was administered after exercise, but using a different technique than Jorres and Magnussen (1990). There were no significant effects of  $\text{NO}_2$  exposure on lung function (single breath nitrogen washout, SRaw, FVC, FEV1) or respiratory symptoms, although a slight increase in SRaw was observed as a result of exercise. Increasing amounts of  $\text{SO}_2$  were administered by successive doubling of the  $\text{SO}_2$  concentration (0.25, 0.5, 1.0, 2.0, 4.0 ppm) at a constant, isocapnic ventilation of 20 L/minutes, maintained for 4 minutes. Specific airway resistance was measured after each step increase in  $\text{SO}_2$  concentration. The concentration of  $\text{SO}_2$  required to increase SRaw by 8 units was interpolated from a dose-response curve of  $\text{SO}_2$  concentration versus SRaw. The dose of  $\text{SO}_2$  was  $1.25 + 0.70$  ppm after air exposure and

1.31  $\pm$  0.75 after NO<sub>2</sub> exposure, indicating no mean change in responsiveness to SO<sub>2</sub>. Only one subject showed a tendency toward increased responsiveness to SO<sub>2</sub> after NO<sub>2</sub> exposure. The contrasting endings in these two studies is somewhat puzzling because the subjects of Rubinstein et al. (1990) were exposed to a higher NO<sub>2</sub> concentration and exercised during exposure. The Jorres and Magnussen subjects appeared to have had slightly more severe cases of asthma and were somewhat older. The modest increase in SRaw induced by exercise in the Rubinstein et al. study may have interfered with the response to SO<sub>2</sub> (i.e., the subjects may have been in a refractory state). Finally, the method of administering the SO<sub>2</sub> bronchoprovocation test (i.e., increased VE at constant SO<sub>2</sub> vs. increasing SO<sub>2</sub> at constant VE) was different and may produce a different response because hyperventilation alone could contribute to the increase in SRaw. Although similar, the two reports using SO<sub>2</sub> challenges are not ideally comparable.

### **Effects of Nitrogen Dioxide on Patients with Chronic Obstructive Lung Disease**

Patients with COPD represent an important potentially sensitive population group. Some of these patients have airways hyper-responsiveness to physical and chemical stimuli. In addition, because of their already compromised lung function, they have much less reserve than people with healthy lung function. The poor distribution of ventilation in patients with COPD may lead to a greater delivery of NO<sub>2</sub> to the segment of the lung that is well ventilated, thus resulting in a greater regional tissue dose. A review of studies by VonNieding and Wagner (1979) summarized previously reported findings. Their observations were that SRaw increased in chronic bronchitis exposed to 2.0 ppm or more NO<sub>2</sub> and that after exposure to 4.0 to 5.0 ppm NO<sub>2</sub>, the partial oxygen pressure (PaO<sub>2</sub>) was decreased and the alveolar-arterial oxygen gradient was widened.

There are several studies of NO<sub>2</sub> exposed asthmatics in which airway responsiveness was evaluated using cholinergic agonists (carbachol, acetylcholine, methacholine). Subjects were exposed to 0.1 to 0.2 ppm NO<sub>2</sub> in five such studies. Of these, both Hazucha et al. (1983) and Roger et al. (1990) found no significant change in group mean response to challenge from methacholine. Ahmed et al. (1983) reported a trend for airway responsiveness to carbachol to increase after a one hour exposure to 0.1 ppm NO<sub>2</sub>, but the trend was not significant ( $p = 0.07$ ). Nevertheless, some subjects appeared to be more responsive than others. Orehek et al. (1976) reported that 13 of 20 subjects exposed to 0.1

ppm NO<sub>2</sub> experienced an increased airway responsiveness to carbachol. In these 13 subjects, the mean challenge dose decreased from 0.66 to 0.36 mg. However, in seven "non responders", the dose of 0.36 mg remained unchanged. A number of questions have been raised about the analytical approach used in this study. Kleinman et al. (1983) also evaluated airway responsiveness to methacholine after a two hour exposure to 0.2 ppm NO<sub>2</sub>. The methacholine dose required to cause a 10% drop in FEV1 decreased from 8.6 to 3.0 mg. As a group, these studies appear to suggest that some individuals, if not a subgroup of asthmatics, may experience increased airway responsiveness after NO<sub>2</sub> exposure. It might be anticipated, when there is a trend for a response at a low concentration, that exposure to increased concentrations would tend to confirm the trend by producing a less equivocal response. Mohsenin (1987a) found a significant decrease in the dose of methacholine required to produce a 40% decrease in flow at 40% of VC on a partial flow volume curve; the PD40 decreased from 9.2 after air to 4.6 after exposure to 0.5 NO<sub>2</sub> for one hour. On the other hand, at both 0.3 and 0.6 ppm for 110 minutes, Roger et al. (1990) found no difference in airway responsiveness to methacholine. Morrow and Utell (1989) also found no change in airway responsiveness to carbachol after a 3.75-hour exposure to 0.3 ppm. These differences cannot be explained either on the basis of NO<sub>2</sub> concentration or total NO<sub>2</sub> dose because the total dose in the Mohsenin (1987a) study was lower than either of the other two studies.

Histamine challenges have also been used in three studies following NO<sub>2</sub> exposure. Two studies by Bylin et al. (1985, 1988), at NO<sub>2</sub> concentrations ranging from 0.14 to 0.53 ppm, suggest possible increased responsiveness to histamine after a 20- to 30-minutes resting NO<sub>2</sub> exposure. In the first study, 5 of 8 subjects showed an increase in response after a 0.48-ppm exposure, and in the second study, 14 of 20 subjects showed an increase in response after a 0.27-ppm exposure. However, the second, larger study (n = 20) did not confirm the observations (at 0.53 ppm) of the first study and a somewhat more conservative statistical approach (Friedman non parametric test) failed to confirm the significance of these findings. In a preliminary report, Rasmussen et al. (1990) reported their exam of the effects of 3 hrs exposures to 0.1, 0.2, and 0.8 ppm NO<sub>2</sub> on airway responsiveness and to histamine challenge. They found no significant mean change in airway responsiveness among the group. Again, these results suggest that some extrasensitive asthmatics may experience increased airway responsiveness after NO<sub>2</sub> exposure. The inconsistent nature

of findings among these studies and the absence of a consistent dose-response relationship is a problem.

Bauer et al. (1986a), Linn et al. (1986), and Avol et al. (1988, 1989) have examined the effects of NO<sub>2</sub> exposure on airway responsiveness to cold air inhalation. Bauer et al. (1986a) found an increase in cold air airway responsiveness after a 30-minutes exposure to 0.30 ppm NO<sub>2</sub>. The airway responsiveness was expressed as the quantity of respiratory heat loss required to produce a 10% drop in FEV1; this averaged 0.83 kcal/min after air exposure and 0.54 kcal/min (p < 0.05) after NO<sub>2</sub> exposure, indicating an increase in airway responsiveness. Linn et al. (1986) found no change in airway responsiveness to cold air after one hour exposures to 0.3ppm, 1.0ppm, or 3.0 ppm NO<sub>2</sub> in a group of 21 asthmatics.

Avol et al. (1988) found a trend for a group mean increase in airway responsiveness to cold air after 0.3 ppm, but not after 0.60 ppm, NO<sub>2</sub> exposure; this increased response was observed in only 11 of the 29 subjects at 0.30 ppm. In a study of young asthmatics, also exposed to 0.30 ppm NO<sub>2</sub> for 1 hour, Avol et al. (1989) found no mean change in cold air airway responsiveness. Indeed, only 12 of 33 subjects demonstrated a change in cold air airway responsiveness in the direction indicative of increased responsiveness. Again, these cannot be explained on the basis of NO<sub>2</sub> concentration or total NO<sub>2</sub> exposure dose because both were lower in the Bauer et al. (1986a) study, where a significant change in the airway responsiveness was observed. Comparison of the studies indicates that the Bauer et al. (1986a) study was shorter, included less exercise, and utilized a mouthpiece exposure system.

The effects of NO<sub>2</sub> on airway responsiveness to a specific antigen have been examined in only two studies. Ahmed et al. (1983a) reported no increase in airway responsiveness to ragweed antigen in a group of allergic asthmatics following 60 minutes of exposure to 0.1 ppm NO<sub>2</sub>. Orehek et al. (1981) found no change in airway responsiveness to grass pollen in a group of allergic subjects (including three asthmatics) after a 60-minutes exposure to 0.11 ppm NO<sub>2</sub>. One of the problems in this kind of analytical exercise is that it is often difficult to distinguish between a negative and a no-change situation (i.e., it is less likely that airway responsiveness would decrease from its baseline level than increase). Of the 105 subjects exposed to <0.20 ppm, the overall data indicated 67 subjects with increased airway responsiveness and 38 with decreased airway responsiveness. Similar ratios were observed for exercise and rest exposures. For the studies of exposure to 0.20 to 0.30 ppm,

using all types of challenges, airway responsiveness increased in 96 subjects and decreased in 73. For studies involving exercise during the exposure, airway responsiveness increased in 71 and decreased in 65. However, both studies involving resting exposure showed significant increases in airway responsiveness, whereas only two of nine studies using exercise exposures were significant. In the resting studies, airway responsiveness increased in 25 subjects and decreased in 8 subjects. These studies also were of shorter duration (30 minutes) than many of the exercise studies. Airway responsiveness increased in 29 and decreased in 13 subjects in studies of 30-minutes duration, whereas there were 41 increases and 53 decreases in exposures lasting 60 minutes or longer.

At concentrations greater than 0.30 ppm, the overall total indicated 48 increases and 33 decreases in airway responsiveness. For resting studies, 24 subjects had increased airway responsiveness and only 9 showed decreased airway responsiveness (5 did not change); whereas 23 increased and 24 decreased in the exercise studies.

The studies in which the change in airway responsiveness was assessed after NO<sub>2</sub> exposure are presented according to whether or not exercise was involved in the exposure. The data are presented as the fraction of the total number of subjects with increased airway responsiveness. The increase in airway responsiveness does not appear to be associated with any particular type of airway challenge. The overall percentage of increased airway responsiveness in NO<sub>2</sub>-exposed subjects was 59 %. This is accounted for almost entirely by the resting studies, with an overall percentage of 69% ( $p < 0.01$ ) (106 increased and 48 decreased), because, in the exercising studies, responses were about equally balanced between increased and decreased responsiveness (104 increased and 96 decreased). There was a trend ( $p < 0.05$ ) for a slightly larger percentage (=75%) of subjects to have increased airway responsiveness after NO<sub>2</sub> exposure when the exposure is performed both under resting conditions and at concentrations above 0.20 ppm. In fact, of the six studies reporting a significant response (Kleinman et al., 1983; Bauer et al., 1986a; Bylin et al., 1988; Jorres and Magnussen, 1990; Mohsenin, 1987a; Bylin et al., 1985), four were resting exposures and, in four, the exposure duration was 30 minutes or less. The implication of this trend is unclear because the brief duration and low ventilation during exposure indicate that the NO<sub>2</sub> exposure dose in these studies is relatively low. If this trend is real, some interesting hypotheses could be generated. Is it possible that exercise during exposure

somewhat interferes with the mechanism causing increased airway responsiveness? It is known, for example, that repeated exercise induces a refractory state such that the subject is less sensitive to exercise-induced bronchoconstriction (Edmunds et al., 1978;). In many cases of NO<sub>2</sub> exposures involving exercise, repeated bouts of exercise were performed during exposure, which could possibly have made the subjects refractory to the effects of NO<sub>2</sub>. During exercise, the responsiveness to methacholine is reduced substantially (Inman et al., 1990) and exercise causes a more rapid reversal of methacholine-induced bronchoconstriction than occurs at rest (Freedman et al., 1988).

### **Comparison Of Subjects With Increased Airway Responsiveness**

The data provide for a possible biphasic response to NO<sub>2</sub> causing hyperresponsiveness of airways at low exposure doses, (e.g. causing mast cell degranulation, Sandstroem et al., 1990a), yet producing a reversal of this response at higher exposure doses, possibly through a direct relaxing effect on airway smooth muscle. Nitrates formed in the lungs of NO<sub>2</sub> exposed animals (Postlethwait and Mustafa, 1981) may have a direct relaxing effect on smooth muscle, including bronchial smooth muscle. In healthy subjects, an increase in airway responsiveness clearly occurs at higher NO<sub>2</sub> concentrations (Beil and Ulmer, 1976; Frampton et al., 1991; Mohsenin, 1988). In healthy subjects at all concentrations, there were 37 airway responsiveness increases and 23 airway responsiveness decreases. At greater than 1.0 ppm, there were 23 increases and 6 decreases, that is, a ratio of 0.79 ( $p < 0.01$ ).

### **Effects Of Nitrogen Dioxide Exposure On Blood, Urine, And BAL Biochemistry**

The effects of NO<sub>2</sub> on the constituents of bronchioalveolar lavage (BAL) fluid, blood, and urine have been examined, both *in-vivo* and *in-vitro*. The general purpose of these studies has been to examine mechanisms of pulmonary effects or to determine NO<sub>2</sub> induced alterations in body fluids that could potentially result in systemic effects. Investigations are directed to determining effects of NO<sub>2</sub> on levels of serum enzymes and antioxidants, as well as direct effects on red blood cells and hemoglobin. Studies of the effects of NO<sub>2</sub> on airway lining fluids have focused on changes in alpha-1-antitrypsin levels. Potential effects of NO<sub>2</sub> on collagen metabolism have been investigated by examining urinary excretion of collagen metabolites.

### **Biochemical Effects in Blood**

Chaney et al. (1981) examined the effects of 0.20 ppm NO<sub>2</sub> on various blood parameters in 19 healthy subjects exposed for two hours while exercising intermittently. A control group of 15 subjects was exposed to clean air. They observed a significant increase in glutathione (GSH) levels after exposure. None of the other blood parameters (red blood cell GSH reductase, 2,3-diphosphoglycerate, methemoglobin, vitamin E, immunoglobulin, and complement C3) were changed significantly. The significance of the response reported in this study appears to be the result of a difference between the control group and the exposure group in general (different subjects were used in each group). The changes in GSH were small and were within the healthy range, with the average baseline level of GSH being approximately 38.5 mg/dl. The post exposure average of the air group was 36.4 mg/dl  $\pm$  1.35 (standard error of the mean [SEM]) and of the NO<sub>2</sub> groups was 40.3  $\pm$  1.19 (SEM) mg/dl. The authors suggested that the increased level of GSH may be in response to oxidation of hemoglobin to methemoglobin by NO<sub>2</sub>. However, Gohil et al. (1988) have recently demonstrated substantial decreases in GSH levels during prolonged submaximal exercise, which was followed by elevated GSH levels in the post-exercise period; GSH levels varied from 0.15 mM during exercise to 0.6 mM three days post exercise, varying about a baseline level of approximately 0.4 mM. It is not clear to what extent the observations of Chaney et al. (1981) may have been confounded by this exercise effect. It should be noted that Posin et al. (1978) found no association between NO<sub>2</sub> exposure (1 ppm for 2.5 h) and GSH levels, although there were apparent changes in blood biochemistry including increased levels of GSH reductase. However, it is not clear from the Posin et al. (1978) study that any of the observed "effects" can be attributed to NO<sub>2</sub> exposure; there was no concentration-response relationship, effects were not reproducible from concentration to concentration, and similar effects were seen with clean air exposures.

In vitro exposure of human blood to high levels of NO<sub>2</sub> (6 and 45 ppm) results in methemoglobin formation (Chiodi et al., 1983). However, Borland et al. (1985) were unable to demonstrate increased methemoglobin levels in smokers exposed to high NO levels from cigarette smoke. Methemoglobin is also formed during in vitro exposure to NO (1,000 ppm) (Chiodi and Mohler, 1985). However, these observations do not have relevance to the potential effects of ambient NO<sub>2</sub>.

### Bronchoalveolar Lavage Fluid Biochemistry

Mohsenin and Gee (1987) have reported that subjects exposed to 3 to 4 ppm NO<sub>2</sub> for three hours had a 45% decrease in the activity of alpha-1-protease inhibitor ( $\alpha$ -Pi), the major lung protease inhibitor of the enzyme elastase. These levels were measured in BAL fluid obtained 3.5 to 4 hrs after exposure. Alpha-1-protease inhibitor is "important in protecting the lung from proteolytic damage, particularly from the elastase of neutrophils." The mean elastase inhibitory capacity decreased from 95  $\pm$  12% in the air group to 55% in the NO<sub>2</sub>-exposed group. (due to analytical impurities in the standard, the 95% inhibition measured in the air-exposed group was presumed equivalent to 100%, thus the 45% difference). The authors noted that even a 50% reduction in the genetic biomarker "cYiPI" activity is not associated with an increased risk of emphysema. However, reduction in protease inhibition could result in connective tissue damage and could conceivably be important in individuals with an  $\alpha$ -1-antitrypsin deficiency.

Johnson et al. (1990) also examined the response of  $\alpha$ -Pi, to *in-vivo* NO<sub>2</sub> exposure in a group of 24 healthy nonsmokers. The subjects were exposed to either 1.5 ppm NO<sub>2</sub> for three hour or to a variable concentration consisting of a baseline level of 0.05 ppm NO<sub>2</sub> with three 15-minutes "peaks" of 2.0 ppm. (Frampton et al., 1989b). Bronchoalveolar lavage was performed 3.5 hours after exposure and the fluid was frozen for subsequent analysis. The functional activity of cYiPI was taken to be the elastase inhibitory activity corrected for the concentration of cYiPI determined by immunoassay. Neither the levels of  $\alpha$ -Pi as determined by immunoreactivity, nor its functional activity were significantly changed by NO<sub>2</sub> exposure.

The different findings by Johnson et al. (1990) and Mohsenin and Gee (1987) with regard to a1PI activity may be accounted for by the considerably larger (about two- to threefold) exposure levels in the latter study. Furthermore, different methods were used to handle the BAL fluid and to quantify (cYiPI concentrations in the two studies. As discussed by Mohsenin and Gee (1987), there appears to be a large range of cYiPI activity that is compatible with lung health, and there is broad range of activity of  $\alpha$ 1A (alpha 1 antitrypsin) in relation to its concentration. The importance of small changes in cYiPI is not clearly established, and therefore, the usefulness of changes in cYiPI activity as a marker of NO<sub>2</sub> exposure will require additional research.

## Urine Biochemistry

Muelender et al. (1987) studied healthy males exposed to 0.6 ppm NO<sub>2</sub> for four h/day on three consecutive days to examine the possibility that NO<sub>2</sub> exposure caused diffuse pulmonary injury. They used hydroxyproline excretion as a marker of increased collagen catabolism or connective tissue injury. Subjects had no residential NO<sub>2</sub> exposure, no allergies or infections that might have produced inflammatory responses, and were minimally exposed to environmental tobacco smoke. Despite controlling for these potentially confounding variables, the authors observed no significant changes in hydroxyproline excretion as a result of NO<sub>2</sub> exposure, either immediately or for up to 9 days after exposure.

Frampton et al. (1989a) studied two groups of healthy subjects exposed to NO<sub>2</sub> under two different protocols that had the same concentration x time (C x T) product. One group was exposed continuously for 3 h to 0.60 ppm and the other was exposed to a background level of 0.05 ppm with three "spikes" of 2.0 ppm for 15 minutes each. The C x T product for each of these two protocols was the same. The major aims of this study were to test the hypothesis that the ability of alveolar macrophages to inactivate influenza virus was reduced by NO<sub>2</sub> exposure, and to examine the possibility that a series of peak exposures would cause more impairment than a constant concentration. Healthy, healthy nonsmokers without a history of airway hyperresponsiveness or of recent upper respiratory infection were exposed to both air and NO<sub>2</sub> in random sequence. Exposures included six 10-minutes exercise periods, coinciding with the "spikes" in the second protocol. There were no significant effects of these exposures on spirometry or plethysmography under either protocol. Alveolar macrophages obtained by BAL were tested in vitro for their ability to inactivate influenza (A/AA/Marton/43 Hind) virus and for the in vitro production of Interleukin-1 (IL-1) by virus-exposed macrophages. Interleukin-1 is an important proinflammatory protein produced by macrophages that performs a number of functions, including induction of fibroblast proliferation and activation of lymphocytes, and is chemotactic for monocytes during the immune response to infection. There were no differences in total cell recovery, viability, or differential cell counts between air- and NO<sub>2</sub>-exposed samples for either protocol. There was a trend ( $p < 0.07$ ) for less effective inactivation of virus by macrophages obtained from subjects exposed continuously to 0.60

ppm NO<sub>2</sub>. This trend was due to the responses of only four of the nine subjects. The macrophages harvested from these four subjects also showed an increase in IL-1 production not seen in macrophages from the other subjects. No effects of virus inactivation were seen in the subjects exposed to the 2.0-ppm spikes.

The results of this study were not statistically significant since it had relatively little power to detect any effect. The findings are suggestive that further work is necessary to test the hypothesis that NO<sub>2</sub> may influence host defense mechanisms in humans.

Frampton et al. (1989b), previously discussed, analyzed the protein content of BAL fluid obtained from NO<sub>2</sub> exposed subjects at either 3.5 or 18 hrs post exposure. Three different exposure protocols were used: three hour exposure to 0.60 ppm or 1.5 ppm NO<sub>2</sub> or a three h variable concentration exposure where three 15-minutes "peaks" of 2.0 ppm were superimposed on a background of 0.05 ppm NO<sub>2</sub>. Exposures included 10 minutes of exercise during each half-hour of exposure. There were no significant changes in pulmonary function or respiratory symptoms observed after NO<sub>2</sub> exposure. Airway reactivity, assessed by carbachol inhalation, was increased after the 1.5 ppm NO<sub>2</sub> exposure (Frampton et al., 1991). Two groups of subjects were exposed to 0.60 ppm so that BAL could be obtained either at 3.5 or 18 hours post exposure. Analysis of BAL fluid obtained 3.5 hours after a 0.60-ppm exposure showed an increase in alpha-2macroglobulin ( $\alpha$ 2-M), a regulatory protein that has antiprotease activity and immunoregulatory effects. The observed increase in  $\alpha$  2-M appears to be transient; no change was seen at 18 h post exposure nor was any change observed at a higher NO<sub>2</sub> concentration (1.5 ppm). Further information appears to be necessary to establish the implications of this finding.

### **Effects Of NO<sub>2</sub> On Macrophage Function In NO<sub>2</sub>-Exposed Humans**

The effect of in vitro exposure to NO<sub>2</sub> on alveolar macrophages harvested by BAL was examined by Pinkston et al. (1988). Fifteen healthy adults underwent BAL to provide macrophages for culture. After an 18-hours incubation, the cells were exposed to 5, 10, or 15 ppm NO<sub>2</sub> or 5% carbon dioxide as a control for an additional 3 hours. Following the exposure, some of the cells were incubated for an additional 24 hours, and cell-free supernatants were then obtained for analysis of neutrophil chemotactic factor (NCF). Other macrophage cultures were incubated for 24 hours with influenza virus and the supernatant was then obtained for analysis of IL-1. There were no changes in macrophage viability,

determined by trypan blue exclusion, in cells exposed to any of the three  $\text{NO}_2$  concentrations. There were no changes in release of NCF in any of the  $\text{NO}_2$ -exposed cell cultures. Furthermore,  $\text{NO}_2$  exposure did not impair the ability of cells to release NCF after stimulation with activated zymosan. Nitrogen dioxide exposure did not stimulate release of IL-1 from exposed macrophages. Influenza virus stimulated the release of IL-1, but there were no significant differences between  $\text{NO}_2$ -exposed and air-exposed macrophage cultures. Therefore,  $\text{NO}_2$  exposure triggered neither the release of NCF, which would attract neutrophils to the airways, nor the release of IL-1, which activates lymphocytes (among other functions). Equally important,  $\text{NO}_2$  exposure did not impair the ability of macrophages to produce either IL-1 or NCF in response to conventional stimuli. Sandstroem et al. (1989) exposed a group of 18 healthy nonsmokers to 2.25, 4.0, and/or 5.5 ppm ( $n = 8$  in each concentration group) for 20 minutes of moderate exercise ( $VE = 35 \text{ L/min}$ ) in a chamber. Bronchoalveolar lavage was performed at least 3 weeks before and 24 hours after each exposure. Increased levels of mast cells in BAL fluid were observed after all  $\text{NO}_2$  exposures. Increased levels of lymphocytes were observed only at the two higher concentrations.

In order to determine the time course of this response, Sandstroem et al. (1990) exposed 32 subjects to 4 ppm  $\text{NO}_2$  for 20 minutes, including 15 minutes of mild exercise, and then performed BAL at 4, 8, 24, or 72 hours post exposure (in four different groups of eight subjects). Increased levels of mast cells and lymphocytes were observed at 4, 8, and 24 hour, but not at 72 hour post exposure. There was no change in macrophage numbers nor in albumin concentration in BAL fluid. Eosinophils, neutrophils, and epithelial cell counts were not altered as a result of  $\text{NO}_2$  exposure. Unpleasant odor and mild nasopharyngeal irritation were typical symptoms. There were no changes in spirometry. The observation of increased numbers of mast cells appears to be unique to this study, although other investigators (Frampton et al., 1989a,b) may not have looked for changes in mast cell numbers. The authors considered increased numbers of mast cells and lymphocytes to represent a nonspecific inflammatory response.

Rasmussen et al. (1992) studied 14 healthy nonsmoking adult subjects exposed to 2.3 ppm  $\text{NO}_2$  and to clean air for five hour with a one-week interval between exposure. Indications of a decrease in alveolar permeability were observed after the  $\text{NO}_2$  exposure. The results support the assumption that a delayed response is a feature of the human

response to  $\text{NO}_2$  and stresses the importance of an extended period of observation in future  $\text{NO}_2$  exposure studies.

Three recent studies examined the effects of multi-hour exposures to 1 to 2 ppm  $\text{NO}_2$  on lavaged cells and mediators. Devlin et al. (1992) studied healthy subjects exposed to 2.0 ppm  $\text{NO}_2$  for four hours with alternating 15 minutes periods of rest and moderate exercise. One of the main findings after  $\text{NO}_2$  exposure was a threefold increase in polymorphonuclear leukocytes (PMNs) in the first lavage sample representing predominantly bronchial cells and fluid. In addition, macrophages recovered from the predominantly alveolar fraction showed a 42 % decrease in ability to ingest (phagocytosis) *Candida albicans* and a 72 % decrease in release of superoxide anion. Frampton et al. (1992) exposed exercising subjects to 2.0 ppm  $\text{NO}_2$  for six hours. Bronchioalveolar lavage was performed either immediately or 18 hours post-exposure. There was a modest increase (< twofold) in PMN (polys) levels in lavage fluid, but no change in lymphocytes. Alveolar macrophage production of superoxide anion was not altered in these subjects. These two studies suggest that  $\text{NO}_2$  exposure may induce a mild bronchial inflammation and may also lead to impaired macrophage function. Jorres et al. (1992) examined both healthy and asthmatic subjects exposed to 1 ppm  $\text{NO}_2$  for three hours, but observed no changes in cells or mediators in BAL fluid or in the appearance of bronchial mucosal biopsies after this exposure. Neither macrophage function nor a specific bronchial washing were examined in this study.

Boushey et al. (1988) studied five healthy volunteers exposed to 0.60 ppm  $\text{NO}_2$  on four days over a six-day period. Exposures lasted two hours each and included alternating 15-minutes periods of rest and exercise (VE Z 30 to 40 L/min). On the final (fourth) day of  $\text{NO}_2$  exposure, venous blood samples were obtained and a BAL was performed. Baseline BAL and pulmonary function data were obtained on a separate occasion. There were no effects of repeated  $\text{NO}_2$  exposure on pulmonary function ( $\text{SR}_{\text{aw}}$ , FVC, FEVI) or respiratory symptoms. Following the fourth day of  $\text{NO}_2$  exposure, a slight increase in circulating (venous blood) lymphocytes was observed ( $1792 \pm 544/\text{mm}^3$  post- $\text{NO}_2$  vs.  $1598 \pm 549/\text{mm}^3$  baseline). The only change observed in BAL cells was an apparent increase ( $p < 0.04$ ) in natural killer (NK) cells from  $4.2 \pm 2.4\%$  (baseline) to  $7.2 \pm 3.1\%$  (post- $\text{NO}_2$ ). The authors expressed reservations that the apparent increase in NK cells may have been an artifact of the cell separation process. Interleukin-1 and tumor necrosis factor levels in BAL

fluid were not detectable. Tumor necrosis factor is another proinflammatory protein that, among other activities, promotes adherence of PMNs to endothelial cells and enhances their phagocytic activity.

Sandstroem et al. (1990) studied a group of eight healthy nonsmokers exposed to 4.0 ppm NO<sub>2</sub> for 20 minutes/day (moderate exercise, VE Z 35 L/min) on alternate days over a 12-day period (seven exposures total). Bronchoalveolar lavage was performed 2 weeks before the first exposure and 24 h after the last exposure. The first 20 ml of BAL fluid was treated separately and presumed to represent primarily bronchial cells and secretions. After NO<sub>2</sub> exposure, there was a reduction in numbers of macrophages in the bronchioalveolar portion, although on a per cell basis, alveolar macrophage phagocytic activity was increased. There were decreased numbers of mast cells in the bronchial portion of the lavage fluid. In addition, there were reduced numbers of T-suppressor, B lymphocyte, and NK cells in the alveolar portion of the BAL fluid compared to the baseline lavage. These observations contrast with those seen by Sandstroem et al. (1989) after single NO<sub>2</sub> exposures, suggesting some alteration in bronchial and alveolar cell populations after repeated NO<sub>2</sub> exposure. The most obvious difference between Sandstroem et al. (1990b) and Boushey et al. (1988) is the higher NO<sub>2</sub> concentration and the longer duration of the former study. Further work is necessary to confirm these observations, to determine the time course of response to repeated exposure, and to determine the NO<sub>2</sub> exposure dose necessary to invoke modification of bronchioalveolar cell populations.

## B. EFFECTS OF NO<sub>2</sub> ON HEALTHY HUMAN RECEPTORS: RECENT DATA

### *In-vitro Effects Of NO<sub>2</sub> On Bronchial Tissue*

An *in-vitro* study exposed an isolated human bronchus to a steady stream of 1.0 or 2.0 ppm of NO<sub>2</sub> or to clean air for 30 minutes duration to identify any change in contractile response to carbachol, histamine or substance P. The results of this study indicates that the 1.0 or 2.0 ppm level of exposure increased the contractile response in isolated human bronchi when subsequently challenged to histamine, carbachol or substance P (Abdellaziz, 1992). The data suggests bronchial smooth muscle is sensitive to the effects of NO<sub>2</sub> exposure, however, isolation of this tissue, may mask the true response of the tissue to exposure when other physiological and biochemical processes are present. As shown in

other studies, clean cold air alone has the ability to alter tissue responsiveness, and is not demonstrated in-vitro.

### **Biomarkers of NO<sub>2</sub> Exposure**

In healthy human volunteers, in rats and during *in-vitro* studies, Maples et al., (1991) found a NO/Heme complex marker by electron spin resonance that indicates a biomarker of nitrogen dioxide exposure is consistent, in humans, experimental animals or in-vitro. Humans were exposed to 1.5 or 4.0 ppm NO<sub>2</sub>, for 20 minutes every second day, for six exposures. Bronchioalveolar lavaged fluid (BALF) cells were analyzed for the biomarkers. They found linear relationships between the marker intensity of the electron spin resonance and the exposure concentration. All eight patients responded at the 4.0 ppm exposure, and 5/8 responded to a 1.5 ppm exposure (Maples et al., 1991). Although useful for identifying NO<sub>2</sub> exposure, the marker is not, necessarily indicative of toxicity or an adverse effect.

### **Effects of NO<sub>2</sub> on Mucociliary Tissue**

The physiological effects of NO<sub>2</sub> exposure can act upon different tissues along the respiratory tract, but their meaning may be insignificant. Seven adult non-smoking men, were exposed twice, to clean air or 2.0 ppm NO<sub>2</sub> for four hrs, three weeks apart. The authors examined nasal tissue under the electron microscope and found qualitative and quantitative evidence in six of seven samples, that the luminal borders of ciliated cells were altered ultrastructurally. Multiple ciliary axonemes were present together with vesiculations of the luminal borders of the ciliary membranes. This pattern was less common among those who were exposed to clean air. However, the findings were not widespread, nor were these responses considered minimally adverse effects since they had not altered mucociliary function in the healthy humans.

### **Effects of NO<sub>2</sub> Exposures on Pulmonary Response**

Pulmonary function tests are valuable indicators of the physiological state of the lungs. However, if the air volumes, ability to ventilate, and gas exchange have been seriously compromised, there has been a serious alteration to that organ. Emphysema and loss of contractility occurs from susceptibility to the effects of smoke; exposure to asbestos and parenchymal scarring also causes change. Numerous events can act to cause these

changes. When measuring changes among healthy or non-healthy humans, pulmonary function may be too large a gauge, when examining the effects of low level, short term exposures. If a pulmonary function examination indicates a serious loss of respiratory ability, the damage has occurred. The healthy individual may be unlikely to demonstrate any ill effects, or the effects may be so trivial as to be undetectable.

However, in an asthmatic, the tissue has been sensitized and may be hyperresponsive when in contact with various materials. Ozone and sulfur dioxide are direct irritants causing bronchoconstriction. Ozone has also been shown to induce non-specific and allergen induced airway hyper-reactivity that provokes airway inflammatory response, or by a direct oxidation effect on tissue. Airborne chemicals can also trigger the immune system, and enhance the IgE response to an allergen (Wardlaw 1993). Thus the effects may be increased risk or induction of an asthmatic condition in a susceptible receptor, or no effect. The data below describes the published, known effects of NO<sub>2</sub> exposure on subjects with pulmonary disease as well as in healthy individuals.

### **Effects on NO<sub>2</sub> Pulmonary Response in Athletes**

Kim et al., (1991) exposed a group of nine healthy athletes, 18-23 years of age, to filtered air and to 0.18 and 0.30 ppm of NO<sub>2</sub> for 30 minutes, while exercising on a treadmill. Results of analysis of indicate there were no changes detected in any of the parameters of pulmonary function at either exposure concentration. Competitive athletes are among the healthiest of humans and possibly because of their finely tuned physiology, detecting distinct changes and any adverse effects of NO<sub>2</sub> may have been impossible. As would be expected, the very low levels of exposure are unlikely to elicit any serious effects. The probabilities are that lung function (FEV, FVC, et.) and capacity will be well above the baseline of the average healthy non-athlete humans, and unlikely to be observed or detected. Using Haber's Law, and the above exposures, the resulting concentration-duration values for a no effect level would be 5.4 ppm\*minutes, (30 minutes x 0.18 ppm) and 9.0 ppm\*minutes, (30 minutes x 0.30 ppm), for Kim's healthy young athletes.

### **Detecting Significant Responses From NO<sub>2</sub> Exposures**

When the biological values of a human organism are measured and clearly outside the boundaries of accepted clinical norms, or where organ function is compromised, or when a

recognized indicator of organ dysfunction is present, an adverse effect or serious adverse response can be clearly identified. It is likely that in order to determine an adverse effect or response from low levels of airborne chemical exposures, such as NO<sub>2</sub>, subtle effects and changes that may occur may not be detected and identified as an adverse response. Accordingly the cellular and/or biochemical changes require critical examination in order to determine where or at what point the response is considered an adverse response. Even then, cellular and biochemical activity in a human body is so very dynamic, and so highly adaptive, that describing subtle changes such as altered cell populations or enzyme levels, as an adverse effect remains a difficult, if not impossible task.

### **Effects of NO<sub>2</sub> Exposure on Bronchial Cell Populations**

Eighteen normal, healthy non-smokers were exposed to 2.25, 4.0 and 5.5 ppm levels of NO<sub>2</sub> for 20 minutes, while performing exercise. The bronchioalveolar lavage fluid (BALF) cells were examined 24 hours after the exposure. In all cases the, inflammatory cell response was observed among all groups (Sandstroem et al., 1991). There were increases in mast cell concentrations at all levels of exposure, and appeared to be dose related. Mast cells are typically early responders to an insult and initiate the damage control and repair process. Increased lymphocyte numbers were observed but only in the individuals exposed at the higher exposure levels of 4.0 ppm and 5.5 ppm. However, taking measurements at 24 hours after the higher exposures during exercise, one would expect to find the presence of inflammatory reaction lymphocytes. These cells are however, latecomers of the inflammatory response, suggesting damage repair and control is active. It may also suggest a slightly higher significance to the higher level of stimulus since they arrived as a response to both higher exposure levels. This exposure point could be interpreted as an adverse response.

The presence of cells in the BALF indicates that a 20 minute exposure to NO<sub>2</sub> at or above 2.25 ppm ( $C \times T = 45 \text{ ppm} \cdot \text{min}$ ) stimulates the inflammatory response in the human organism. However, this level of exposure failed to damage the target organ, sufficient to modify its function and affect the well being of the organism. In these subjects, pulmonary functions were within healthy limits and without observed adverse effects. An inflammatory cell response in these individuals, demonstrates an effect in response to an insult. However, the response is without functional lung damage or any indications of a significant

or irreversible adverse effect. If the late appearance of lymphocytes is considered a significant response, it may be a useful point for decision making. However, this response is not a likely candidate of an adverse effect. Using Sandstroem's values of exposure concentration and duration and Haber's Law, the concentration-duration exposure values for the lowest exposure level and duration that failed to produce a "significant response", was 45.00 ppm\*min (20 minutes x 2.25 ppm) and may be considered a No-Observed Adverse Effect Level (NOAEL) in this group.

The late appearance of lymphocytes was also seen in other exposed humans and was not characterized as an adverse effect. Rubinstein et al., (1991) examined effects of NO<sub>2</sub> exposure on circulating and bronchial lavage lymphocyte phenotypes in five healthy adult non-smokers, exposed to 0.60 ppm NO<sub>2</sub> for 120 minutes, during intermittent light to moderate exercise on four separate days, but within a six day period. The lower NO<sub>2</sub> concentration, with a longer duration of exposure failed to initiate any observed adverse effects. Ventilation rates and volumes increase with exercise so that total dose increases, however, pulmonary function tests indicate no changes, either after the daily exposures or after the 4 day exposure. Levels of circulating lymphocytes increased slightly over baseline, but were statistically non-significant. Proportions of T/B lymphocyte subsets and the large granular cells also remained the same as pre-exposure levels and failed to demonstrate any shift in populations. They concluded that exposures of healthy individuals to 0.60 ppm NO<sub>2</sub> do not cause clinically significant symptoms, changes in airway caliber, or alterations in circulating and BALF cells, nor would it provoke impairment in healthy non-smoking adults. The concentration-duration value for this group was 120 min x 0.60 ppm or 72 ppm\*minutes and the response is in agreement with the 80.00 ppm\*min value shown for Sandstroem's exposure group.

### **Effects of NO<sub>2</sub> Exposure on Young Smokers**

Although the chronic effects of smoking on lung function and tissue are known and have been studied, the effects of NO<sub>2</sub> exposure on current smokers without serious known disease are not well characterized. A study of 3.5 ppm NO<sub>2</sub> exposure for 20 minutes was conducted among young non-bronchitis smokers and non-smokers. The subsequent examination of cell numbers from bronchioalveolar lavage fluid indicated that the smokers showed a higher level of total cells and alveolar macrophages (AMΦ), but a lower level of

lysozyme positive AMΦ. Prior to exposure smokers showed increased levels of AMΦ with reduced phagocytic ability. After exposure the smokers showed increases in AMΦ and neutrophils (Polys) but no lymphocytosis.

Non-smokers, on the other hand, show a typical inflammatory response, due to the higher NO<sub>2</sub> exposure concentration, with an increase in lymphocytes and a mild increase in Polys, but with a subsequent reduction in their phagocytic ability. This level of response signals an alteration that may be considered significant since a system function has been compromised. The reduction of the phagocytic ability of neutrophils in this group signals an adverse response in host defense-immune function. Any receptor unable to develop a neutrophil phagocytic response to a microbial infection would be a serious compromise in host defense function. This level and duration of exposure [C x T; 3.5 ppm NO<sub>2</sub> for 20 minutes] could be considered a significant effect and may be the lowest observed adverse effect level (LOAEL).

The smokers response to exposure demonstrates the presence of a compensatory mechanism to oxidant stress, due to pre-existing airway inflammation. This may be confirmed by the presence of higher levels of AMΦ, and enhanced antioxidant activity shown by higher levels of glutathione, superoxide dismutase, and catalase (Helleday, 1994). The increase in lymphocyte populations among nonsmokers is consistent with previous data above. The smokers lung apparently has an altered response, shown by the variability of cell sub-sets and functions, due to pre-existing modifications induced by smoking.

#### **Effects of NO<sub>2</sub> Exposure on Airways of Healthy Elderly Adults**

Morrow et al (1992) examined the effects of exposure to 0.3 ppm NO<sub>2</sub> for four hours on 20 healthy elderly adults and also on 20 aged matched (49-69 year old) elderly patients with chronic obstructive pulmonary disease (COPD). They found no significant changes in pulmonary function. Seven of the 20 healthy group members had been smokers and were compared statistically with the non-smokers; the former smoker sub-group showed a significantly lower mean FEV<sub>1</sub>.

As discussed earlier, Frampton (1991) has studied the effects on adults with continuous NO<sub>2</sub> exposure to 0.60 ppm (9 subjects), or continuous exposure to 1.5 ppm (15 subjects), for three hours duration, while a group of 15 was exposed to 0.05 ppm NO<sub>2</sub> with intermittent

15 minutes peaks of exposure at 2.0 ppm. All performed exercise to increase ventilation volumes to 40 L/min. Thirty minutes after exposure, groups were challenged with carbachol. The 0.60 ppm exposure group and 0.05 ppm baseline with 2.0 ppm peak exposure group showed no alteration in pulmonary function, nor was airway reactivity or responsiveness affected. The continuous 3 hrs. exposure to 1.5 ppm NO<sub>2</sub> did show a small 3.9% decrease in fixed vital capacity (FVC) and fixed expiratory volume (FEV) (p= < 0.01). Air intake alone caused a 1.5% decrease in FVC and FEV. They concluded that short term low level exposures do not affect airway mechanics in healthy subjects.

Mohsenin (1988), concluded that 18 subjects demonstrated a 40% reduction in airway reactivity from the effects of exposure to 2.0 ppm NO<sub>2</sub> for one h, although no significant changes were detected in lung function tests. The results are in contrast to Frampton, (1991) who also studied subjects exposed to 2.0 ppm for 60 minutes; the total intakes were different due to differing ventilation rates (V<sub>r</sub>). The V<sub>r</sub> was 40 L/min in the Frampton group but only 7 L/min. among the Mohsenin group. Intake was approximately 1.80 mg. for Mohsenin group and was much less than the intakes among the exercising Frampton groups, which was 3.38 mg for the group inhaling 0.60 ppm NO<sub>2</sub>, (0.60ppm= 1.13 ug/L for 180 min x 40 L/min. ventilation rate), or 5.634mg for the group inhaling the 15 minutes peak 2.00 ppm NO<sub>2</sub>, and 8.123 mg for the group inhaling the continuous 1.5 ppm NO<sub>2</sub> exposures. Several possible reasons for the change are; greater levels of baseline reactivity in the Mohsenin group, the higher cutoff point used to choose patients that allowed possible asthmatics into the group. The most reasonable explanation is that the relationship of exposure and response are more complicated than a simple dose response.

In the Frampton group, 2.0 ppm peak concentrations of NO<sub>2</sub> failed to bring on a response while 1.5 ppm for three hours elicited a pulmonary change, suggesting that the duration of exposure appears to play a significant role in possible response as does background or baseline levels to which receptors are constantly exposed.

These data are consistent with many other previous studies, showing the variability among health or in non-healthy subjects, regardless of age. Hackney (1978) examined the effects of exposure to 1.0 ppm NO<sub>2</sub> in purified air, for two hrs among 20 adult male volunteers; 15 non-smokers, 2 smokers and 3 former smokers, performing intermittent light exercise. Effects were assessed by physiological pulmonary responses and a clinical evaluation. The results of examination of subjects from exposure indicate a small marginal

functional loss (1.5%) that was statistically not significant. The authors conclude that short term toxicity of exposure to ambient levels, (1.0 ppm NO<sub>2</sub> for 120 minutes = 120 ppm\*min) appears to be substantially less than that of ozone among healthy people, but that the effects attributable to diseased receptors or in long term exposures cannot be ruled out (Hackney et al., 1978).

### **Effects of Extended Duration Exposure to NO<sub>2</sub>**

Although the typical duration of exposure to NO<sub>2</sub> or from the products of rocket combustion are likely to be less than an hour, the effects from a longer term low level exposure provide valuable informative, in that response to an exposure, indicated by change or progression of cell activity during the inflammatory process, may be charted. Rasmussen et al., (1992) examined the effects of extended duration of exposure to 2.3 ppm NO<sub>2</sub> for 5 hrs among 14 healthy adults (C x T = 690.0 ppm\*min.). Examination of airway resistance failed to show any changes 11 hrs after exposure began. There were no indications of mucous membrane irritation, or decreased lung function during or after exposures, but examination after the 11<sup>th</sup> hour showed a 22% decrease in alveolar permeability. Twenty four hrs after exposure, a 14 % reduction in serum glutathione peroxidase activity was demonstrated. These results suggest a delayed response from prolonged exposure to a level that was below the then current TLV, and the present 1995-96, ACGIH-TWA level of 3.0 ppm. These results indicate that any adverse impact of NO<sub>2</sub> exposure on a healthy adult, may be more a matter of duration of exposure, or possibly the dose rate or the cumulative dose. Studies in rats (Gelzleichter et al., 1992), have shown that acute lung damage is a function of cumulative dose. However, in combined exposures (ozone plus NO<sub>2</sub>), lung damage was a function of peak concentrations. These exposures indicate deviations in Haber's Law and are expected since the combined exposure is the result of synergistic effects.

A study of lung function was conducted on a group of 21 healthy, young non-smoking women, 18-35 years old, in an effort to identify the relationship of effects of ozone exposure and/or exposure to 0.6 ppm NO<sub>2</sub> for two hours by measuring pulmonary function. The exposure included exercise for 15 minutes, alternating with 15 minutes rest for the two hrs period of exposure, (Ventilation rates (V<sub>e</sub>) averaged 34 L/min.). The women underwent two exposures on two different days, each separated by two weeks. Subsequent spirometry

was performed at one hour intervals for the 2 hrs. period. Measurements taken after the exposure failed to demonstrate any changes in airway restrictions or in lung function (Fixed expiratory volume for 1 sec., FEV<sub>1</sub>) tests. There is a lack of any acute or delayed responses when the exposure is of short duration or at low exposure levels. The pre-exposure to NO<sub>2</sub>, followed by ozone (1.7 mg/ml) exposure, enhanced the responsiveness of the airways, and FEV<sub>1</sub> was significantly reduced.

### **C. EFFECTS OF NO<sub>2</sub> ON SENSITIVE HUMAN RECEPTORS: RECENT DATA**

#### **Sensitive Receptors**

Human receptors with a pre-existing pulmonary dysfunction or unusual hypersensitivity such as emphysema, bronchitis, or asthma, immunological atopy, or chronic obstructive pulmonary disease (COPD), extrinsic allergic alveolitis (EAA), and the like are considered sensitive receptors. In addition, neonates and infants, the aged and infirm, are also considered part of the sensitive group. The reason for concern among this group is, any adverse or stressful effect on pulmonary physiology could exacerbate present illness or lead to a severe response, if unattended, or may initiate a disease state due to altered susceptibility. Children, because of the rapid metabolic activity and accelerated growth periods, are also considered a sensitive group as are the aged or infirm due to their slower or reduced rate of metabolic activity and response to an insult.

#### **Effects of NO<sub>2</sub> Exposure on Patients With COPD**

Linn et al (1986) examined pulmonary function response of patients with Chronic Obstructive Pulmonary Disease (COPD) to levels of 0.2, 1.0 and 2.0 ppm of NO<sub>2</sub> for 60 minutes, and did not find any consistent lung dysfunction among the exposed groups. Morrow et al., (1992) examined patients with COPD, who performed light exercise during a four hrs exposure to 0.03 ppm NO<sub>2</sub>. Thirteen men and seven women, all elderly smokers, responded by measured decrements in FVC and FEV<sub>1</sub>. The results suggest responsiveness to NO<sub>2</sub> decreased with the degree of severity of the COPD and the individuals with lung dysfunction are unable to respond in a manner typical of a healthy individual. The response in healthy individuals appears to be a healthy defense mechanism, adapting to the insult. Another group of elderly adults, 15 men and 11 women, 47-69 years old., with chronic respiratory illness, (all were heavy smokers) were exposed

to 0.03 ppm NO<sub>2</sub> for four hours with four 7 minutes exercise periods. Ventilation rates (V<sub>e</sub>) were up at 25 L/min. Hourly symptom reports and FEV measurements indicated no statistically significant differences between those exposed to clean air vs. those exposed to the NO<sub>2</sub>.

### **Effects Of NO<sub>2</sub> Exposure On Asthmatics**

Rogers et al (1992) showed that as little as one 0.03 ppm NO<sub>2</sub> exposure for 30 minutes during an exercise period was sufficient to cause bronchoconstriction among a group of 13 male asthmatics. However, in conducting further tests, they (Rogers et. al.), found that in a group of 21 male volunteers with mild asthma exposed to 0.0, 0.15, 0.30 and 0.60 ppm NO<sub>2</sub> for 75 minutes, with 10 minutes exercise, the group average airway responsiveness that was not significantly different than after exercise in clean air. Only two of 21 showed any increase in responsiveness. In fact, exercise alone in a controlled air exposure, caused substantial decrements in pulmonary function. They concluded that, a study using a single concentration, fails to support the results published by them as well as others.

DeVallia and associates (1994) have measured the effects of NO<sub>2</sub> , alone and mixed with SO<sub>2</sub> on atopic asthma patients, using 0.40 ppm NO<sub>2</sub> and provoked responses with inhaled allergen. NO<sub>2</sub> failed to elicit any response different than that produced by air alone. However, in concert with SO<sub>2</sub>, the decrease in airway responsiveness was highly significant. The combined response appears to be a synergistic effect. A contrary result was previously published by Jorres and Magnussen, (1990) who exposed 14 mild asthmatics to 0.25 ppm NO<sub>2</sub> for 30 minutes on three separate days and found that NO<sub>2</sub> did not increase airway tone, but enhanced airway responsiveness to subsequent hyperventilation of sulfur dioxide (SO<sub>2</sub>). Jorres and Magnussen (1991) conducted another study and confirmed the no effect response found by DeVallia of exposures to 0.25 ppm NO<sub>2</sub>. Eleven mild and stable asthmatic patients were exposed to 0.25 ppm NO<sub>2</sub>, in an effort to determine whether that level had any effects on airway resistance in asymptomatic patients. On two separate days the patients inhaled 0.25 ppm NO<sub>2</sub> or filtered air, for 20 minutes followed by 10 minutes exercise and followed by methacholine challenge one hour after the end of the exercise. The result of measurements indicated that there was no effect. In mild and stable asthmatic patients, the short term exposures to 0.25 ppm NO<sub>2</sub> during rest and exercise did not increase airway responsiveness to methacholine 1 h after exposure.

Tunnicliffe et al.,(1994) conducted studies of FEV on 10 subjects from the effects of domestic exposures to NO<sub>2</sub>, and confirms the fact that airway responsiveness among asthmatics is only minimally affected by the very low levels [100 and 400 parts per billion, ppb] of exposure.

The most recent data by Jorres et al., (1995), noted effects of 1.0 ppm exposure for 180 minutes total or to filtered air. Asthmatic and healthy human subjects were examined. Subjects were exposed on three separate days, each, at least one week apart. Each three hour session consisted of multiple 10 minutes rest and 10 minutes exercise sessions. Lung function spirometry was determined within five minutes at the end of the 20 minutes period. This test was repeated for a total exposure of three hours. Measurements of lung function were made at 2, 10, 20 and 30 minutes after the total three hours exposure, and "borderline" changes noted. One hour after the air or NO<sub>2</sub> exposure, bronchoscopy was performed and cells collected from lavage fluid for counting subsets and production of inflammatory mediators. The results show that healthy individuals were unaffected by this exposure. Asthmatics showed a small drop in Forced Expiratory Volume (FEV1), but differential cell counts of the lavaged fluid failed to show any significant changes. Although levels of thromboxanes and prostaglandins were increased in some subjects, in others levels of these mediators were reduced, but none were significantly changed. On close examination, the numbers of BALF cells among asthmatics showed minimal, reductions due to NO<sub>2</sub> exposure. In fact they were almost identical to those occurring from filtered air. Eosinophils levels were the only cell values to show significant change, ( $p=<0.05$ ); however, eosinophil levels were significantly different in the controlled healthy populations. In spite of the fact that there were no significant alterations of lung function, of any cell populations, or levels of inflammatory mediators, the study authors concluded that, in mild asthmatics, NO<sub>2</sub> is capable of inducing an activation of cells, compatible with enhanced airway inflammation, even though lung function and BALF cell composition are not markedly effected.

### **Review of Studies Of NO<sub>2</sub> Exposure And Toxicity**

Epidemiology studies reported that the young, the aged, and those with cardiopulmonary illnesses were susceptible to the adverse effects of NO<sub>2</sub> exposures (Schneider and Grant, 1982; NAS, 1977). Based upon the results of these reports, those populations were defined as susceptible populations. Subsequently, research studies

focusing on the effects of  $\text{NO}_2$  were given high priority due to  $\text{NO}_2$  levels and the duration that it remains in the air, along with its potential for adverse effects. The earlier laboratory studies of  $\text{NO}_2$  toxicity on humans and animals were subjects of reviews by Lee (1980), and Morrow (1984), and Morrow and Utell (1989). Two early epidemiology studies, (NAS, 1977), indicate adverse effects from exposures of 0.08 and 0.16 ppm; concurrently, they also reported the results of two other studies with lower average  $\text{NO}_2$  exposures, that failed to reveal any association. Clinical studies reviewed by Shy and Love (1980), concluded no significant reductions in pulmonary function at  $\text{NO}_2$  exposures of levels below 1.5 ppm for two hours duration or less in healthy subjects. Others report various responses; enhanced bronchial responsiveness among 13/21 asthmatic subjects exposed to 0.11 ppm  $\text{NO}_2$  (Orehk, 1976), non-significant reductions in pulmonary compliance among 20 healthy and 20 asthmatics or chronic obstructive pulmonary diseased (COPD) patients exposed to 0.5 ppm for 2 hrs (Kerr et. al., 1979), marginal reduction in Forced Vital capacity (Hackney et al., 1978), and no consistent changes in airway resistance lung volumes, oxygen saturation diffusion capacity, or pulmonary mechanics of 1.0 ppm or less during exposure or up to 2 weeks after the exposure (Sackner et al., 1980; 1981).

Subsequent studies indicate that a highly specific response among challenged individuals may be occurring. Ahmed (1983) challenged healthy and asthmatic subjects to 0.1 ppm  $\text{NO}_2$ , for one hrs. One half the healthy group and one half of the asthmatic group responded to carbachol challenge prior to exposure. After the exposure, the same responders within both groups showed an enhanced response. Concurrently, the non-responders of each group failed to respond to challenge after the exposure. Hazucha, et al., (1983; 1994) report the lack of response in pulmonary function in either healthy or asthmatic subjects after 0.1 ppm exposure for one hrs while Kleinman et al., (1983) observed enhanced responses from 0.2 ppm exposure for two hrs, while Linn et al (1985) found no responses of enhanced airway activity among healthy and asthmatic subjects exposed to 4.0 ppm for 75 minutes.

Normal, healthy individuals consistently fail to show any serious ill effects from most low level short term exposures. Individuals with mild asthmatic or COPD conditions have not responded consistently or significantly to the low levels of  $\text{NO}_2$  exposures cited. Most data cited above is in agreement that acute exposures of low level  $\text{NO}_2$  up to 4.0 ppm for one to two hours, does not appear to have any significant effects on the airway mechanics of

young or elderly healthy individuals (Frampton, 1991). Although there are responses among mild asthmatics, they have not consistently responded with a significant increases in airway responsiveness from exposures of 0.12 to 4.0 ppm from between 20 to 120 minutes (Hazucha, 1995; Jorres and Magnussen, 1990). Patients with COPD also have not shown consistent lung function changes with exposures to 0.5, 1.0 and 2.0 ppm for one hour (Linn, 1985).

In the majority of studies, pulmonary function tests have generally been inconsistent. As described above, the variability in methods, the conditions of exposure, the diagnosis of an adverse effect, accuracy in measurements or the imprecision of exposure doses all make these study conclusions uncertain.

#### **4.3 REVIEW OF NO<sub>2</sub> DATA**

Symptoms associated with NO<sub>2</sub> exposure in healthy subjects limited to detection of the odor of NO<sub>2</sub>, and in some cases at surprisingly low concentrations, less than 0.1 ppm (Bylin et al., 1985). A few studies examined in this review noted a significant increase in respiratory symptoms. Sandstroem et al. (1990) noted mild nasopharyngeal irritation after exposure to 4 ppm for 20 minutes. Nitrogen dioxide exposure at sufficiently high concentrations appears to produce changes in lung function in healthy subjects. A number of investigators have reported increased airway resistance after exposure to NO<sub>2</sub> concentrations exceeding 2.5 ppm (Utell, et al., 1980). However, at concentrations of NO<sub>2</sub> between 2 and 4 ppm, some investigators have not observed any NO<sub>2</sub> induced changes in airway resistance or spirometry (Linn et al., 1985b; Mohsenin, 1987b; Mohsenin, 1988; Sandstroem et al., 1990a). At NO<sub>2</sub> exposure concentrations below 1.0 ppm, there is little if any convincing evidence of change in lung volumes, flow-volume characteristics of the lung, or airways resistance in healthy subjects. Nitrogen dioxide is believed to have its primary effect on small airways. However, routine spirometry and airway resistance measurements are not sensitive indicators of small airways function. Thus, the absence of change in these physiological indicators of large airways function at low NO<sub>2</sub> concentrations should not be viewed as evidence that NO<sub>2</sub> has no effects on lung function. Further developments will be necessary to permit sensitive, reproducible, noninvasive evaluation of small airways, the primary site of NO<sub>2</sub> deposition in the lung.

Nitrogen dioxide exposure may or may not initiate increased airway responsiveness in healthy subjects exposed to concentrations in excess of 1.0 ppm. Mohsenin (1987b) and Frampton et al. (1991) reported an increase in airway responsiveness after exposure to 2.0 and 1.5 ppm, respectively. Repeated bouts of airway inflammation may promote deleterious long-term changes in the lung, such as loss of elasticity and acceleration of age-related changes in lung function. However, the development of such responses is only speculative, given the present level of scientific evidence.

Potentially sensitive subjects in the population include children, older adults, patients with asthma or COPD, or individuals who may be unusually sensitive to NO<sub>2</sub> for other reasons. There are insufficient data on children, adolescents, or older adults, healthy or with asthma, to determine their NO<sub>2</sub> responsiveness relative to healthy young adults. At concentrations that may fall within the ambient range (e.g., < 1.0 ppm), the effects of NO<sub>2</sub> on lung function (i.e., spirometry, airway resistance) in asthmatics have tended to be small, and if measured among a few subjects are unreliable for general distribution. For example, Bauer et al. (1986) observed a 4 to 6% decline in FEV in asthmatics exposed to 0.3 ppm NO<sub>2</sub> for 30 minutes. Koenig et al. (1988) reported a 4% decrease in FVC, but no significant change in other spirometry variables, after exposure of adolescent asthmatics to 0.30 ppm NO<sub>2</sub>. On the other hand, several other investigators (Avol et al., 1988; Bylin et al., 1985; Hazucha et al., 1982, 1983; Kleinman et al., 1983; Koenig et al., 1985; Linn et al., 1985b, 1986; Mohsenin, 1987; Roger et al., 1990) have not found any significant changes in spirometry or airway resistance of asthmatics exposed to concentrations < 1.0 ppm. Again, spirometry and airway resistance are not sensitive measures of small airways function, where NO<sub>2</sub> is known to be primarily deposited.

Another important category of sensitive subjects are patients with COPD, who show increased airway resistance after brief exposures to levels greater than 1.6 ppm NO<sub>2</sub> (On Nieding et al., 1977; 1979). During a longer (four hours) exposure, Morrow and Utell (1989) reported decreased (approx. 5%) FVC in COPD patients exposed to 0.30 ppm. Other investigators (Linn et al., 1985a; Kerr et al., 1979) did not find responses in COPD patients even to exposure levels as high as 2.0 ppm. It appears, brief acute exposure to relatively high concentrations of NO<sub>2</sub> (> 2 ppm) will cause bronchoconstriction in some COPD patients; these responses may also be observed with longer exposures to lower concentrations.

A confusing and unresolved issue in the data base are reports of the existence of NO<sub>2</sub> induced pulmonary responses in asthmatics occurring at low but not at high NO<sub>2</sub> exposures. Although small functional responses have been observed in studies from various laboratories, effects are not consistently present and demonstrating reproducibility of responses has been difficult, even within the same laboratory. Furthermore, all responses to NO<sub>2</sub> that have been observed in asthmatics have occurred at concentrations between 0.2 and 0.5 ppm. Changes in lung function or airway reactivity have not been seen even at much higher concentrations (i.e., up to 4 ppm). There is, at present, no explanation for this apparent lack of a concentration-response relationship and it requires clarification. There is a possibility that a portion of the variability in response to NO<sub>2</sub> may be attributed to differences in the severity of asthma. This is a complex issue and has not been studied adequately at this time. In patients with chronic obstructive lung disease, Bauer et al. (1987) and Morrow and Utell (1989) have observed decreased lung function (FVC, FEV1) after exposure to 0.30 ppm for four hours, but Linn et al. (1985) and On Nieding and Wagner (1979) found no effects in COPD patients from short duration exposures below 2.0 ppm. It appears further work is necessary to provide enough information to estimate the concentration-response relationships for NO<sub>2</sub> exposure of asthmatics and COPD patients, who appear to be the sensitive sub-populations.

In several studies of asthmatics exposed to NO<sub>2</sub>, airway responsiveness to a variety of agents has been demonstrated. However, in many other studies using similar experimental exposures, there was no significant change in airway responsiveness. In order to evaluate this apparent dilemma, a meta-analysis was utilized. Without regard to the type of airway challenge, NO<sub>2</sub> concentration, exposure duration, or other variables, the overall trend was for airway responsiveness to increase (59% of 354 subjects increased). This trend was somewhat more convincing for exposures conducted under non-exercising conditions (69% of 154 subjects increased); the excess positive responses were almost entirely accounted for by exposures during resting conditions. The ultimate impact of this trend is unclear, requiring further investigations to verify if there is an interaction with exercise-induced changes in lung function that may obscure changes in airway responsiveness due to NO<sub>2</sub> exposure. Increased airway responsiveness could potentially lead to temporary exacerbation of asthma, possibly leading to increased medication usage or even increased

hospital admissions. The lowest observed effect level for this response appears to be in the 0.2- to 0.3-ppm range.

Several recent studies have examined the possibility that NO<sub>2</sub> could induce a pulmonary inflammatory response and/or alter immune system host defenses. These studies typically include collection of cells and airways fluids from the lung using BAL. In contrast to ozone (O<sub>3</sub>) exposure, NO<sub>2</sub> does not, at the concentrations studied, induce an increase in BAL levels of neutrophils, or eosinophils, the typical markers of inflammation following O<sub>3</sub> exposure. However, Devlin et al. (1992) have reported increased PMN's in bronchial washings. Sandstroem et al. (1990a) observed an increase in mast cells and lymphocytes in BAL fluid, which they attribute to a nonspecific inflammatory response. Boushey et al. (1988) report increases in natural killer lymphocytes in BAL fluid. Macrophage numbers were not increased by exposure, nor did their ability to kill virus appear to have been altered by exposure; however, Frampton et al. (1989a) suggest that, in some subjects, macrophage responses may have been impaired. Rasmussen et al. (1992) observed indications of a decrease in alveolar permeability after exposure to 2.3 ppm NO<sub>2</sub> for five hours. Mucociliary clearance was not altered after NO<sub>2</sub> exposure in the one study in which it was measured (Rehn et al., 1982). Nitrogen dioxide was found to cause a reduction in alpha-antiprotease activity in one study (Mohsenin and Gee, 1987), but not in another (Johnson et al., 1990). Following NO<sub>2</sub> exposure, Frampton et al. (1989b) found an increase in alpha2-macroglobulin, a molecule that has immunoregulatory as well as antiprotease activity. Immunological responses to NO<sub>2</sub> exposure are just beginning to be elucidated and additional research will be required to determine whether these responses have any implications for epidemiologically determined associations between NO<sub>2</sub> exposure and increased respiratory tract infections.

The effects of repeated NO<sub>2</sub> exposure have been examined in two studies (Sandstroem et al., 1990b; Boushey et al., 1988). Boushey et al. (1988) reported only a slight increase (12%) in circulating lymphocytes and a possible increase in natural killer lymphocytes after four two hour exposures to 0.60 ppm. There were no detectable changes in inflammatory mediators. Sandstroem et al. (1990), on the other hand, found decreased numbers of mast cells, macrophages, and lymphocytes in the BAL fluid. Despite the decreased numbers, the phagocytic activity of alveolar macrophages was enhanced. These observations suggest that host defense responses are different after repeated exposure than after a single acute

exposure. More research appears to be necessary to confirm and expand these observations because of the important potential connection between altered host defense responses and increased respiratory infectivity.

In healthy adults, a variety of mixtures of other pollutants with NO<sub>2</sub> have been examined, primarily using spirometry and airway resistance measurements as end points. In general, NO<sub>2</sub> does not appear to cause significant exacerbation of responses to other pollutants. There is no more than an additive response when NO<sub>2</sub> is included in the pollutant mixture. However, further investigation of NO<sub>2</sub> mixtures appears warranted using other biological markers, including measures of epithelial permeability, clearance, airway responsiveness, airway inflammation, and measures that are sensitive to changes in small airways function. In asthmatics, there is a tendency for increased responsiveness to cold air, methacholine, carbachol, and histamine after NO<sub>2</sub> exposure (see previous discussion).

In one study, asthmatics were also more responsive to SO<sub>2</sub> after a previous exposure to NO<sub>2</sub> (Jorres and Magnussen, 1990). In addition to interactions with other pollutants, NO<sub>2</sub> exposure could potentially enhance (or inhibit) responses to other substances, particularly airborne antigens. In two studies (Ahmed et al., 1983; Orehek et al., 1981), the response to grass pollen inhalation was examined in sensitive subjects after exposure to 0.1 ppm NO<sub>2</sub>, but no significant difference in the response after air and NO<sub>2</sub> exposures was observed. Given the increase in responsiveness to non-antigenic substances such as methacholine, histamine, SO<sub>2</sub>, or cold air discussed previously, it may be worthwhile to reexamine this hypothesis using higher NO<sub>2</sub> concentrations or more prolonged exposures.

In humans, short term, acute NO<sub>2</sub> exposures evoke symptoms of strangling in the throat, an irritating cough, breathlessness, retro-sternal pain, vomiting and general complaints of malaise (Meulenbelt et al., 1992). These symptoms, may or may not be present, are transient and generally pass quickly. However, among those exposed to very high concentrations of NO<sub>2</sub>, chronic obstructive pulmonary disease, emphysema, and bronchiolitis obliterans were identified as the response to the exposure (Horvath, 1978).

Lower concentrations provide mixed results, not only in healthy subjects but also in sensitive receptors. Despite publications that NO<sub>2</sub> exposure has been identified as a causative agent of increased airway responsiveness in healthy subjects, the data suggests that the exposure dose, duration, and conditions will produce various responses (Frampton et al., 1991; Rubinstein et al., 1991; Kleinman, 1983; Bauer, 1986; Mohsenin, 1988; Rogers

et al., 1990; Sandstroem, 1991; Bylin, 1992; 1993; Folinsbee, 1992a; 1992b;). However, this review includes a critical examination of the human responses at the cellular and cell product and enzyme levels to elucidate those that are significant or possible adverse responses. Table 4.2 summarizes human studies of two hours or less NO<sub>2</sub> exposure.

**Table 4.2**  
**SUMMARY OF HUMAN EFFECTS STUDIES OF 2 HRS. OR LESS NO<sub>2</sub> EXPOSURE**

DOSE	DURATION	GROUP/EFFECTS	REFERENCE
1.5 ppm	120 min.	N human-no effects	Shy and Love, 1980
0.11 ppm	-	13/21 A- enhanced airway response.	Orehek, 1976
0.5 ppm	120 min.	20 A/COPD. non-sig. drop↓ in pulmonary function.	Kerr et al., 1978. Hackney et al., 1978
1.0 ppm	120 min	A-no sig. Effects	Sackner et al., 1981
0.1 ppm	60 min.	N or A- no observed response	Hazucha, 1983, 1984.
0.12 to 4.0 ppm	20-120 min	N & A No consistent response in airway change	Hazucha, 1995 Jorres/Magnussen, 1990
0.2 ppm	120 min.	Healthy/asthmatics - enhanced ↑ response	Kleinman et al., 1983
4.0 ppm	75 min.	Normal/asthmatics - no change in airway response	Linn et al., 1985
2.0 ≥ ppm	60 min	No change in pts w/ COPD	Linn, 1985.
4.0 ppm	60-120 min	N - no effects	Frampton, 1991

N= normal healthy humans

A= asthmatics

COPD = chronic obstructive pulmonary disease.

Pts. = patients

↓,↑ = decrease or increase

#### 4.4 DISCUSSION OF DATA AND CONCLUSION

Nitrogen dioxide has been reported to possibly affect the pulmonary tract tissue, and the indications are that the effects of NO<sub>2</sub> occur in the bronchioalveolar airways. The sum and substance of the database is confusing and contradictory. However, there is little evidence to suggest that there are any significant effects from exposure among normally healthy subjects from short term low level exposures. The data for responses of less than one hour exposure is essentially non-existent. Concurrently, available data for one h exposure is equivocal. Any increases in airway responsiveness occurring among healthy,

nonsmoking subjects were generally in excess of 1.0 ppm and of extended duration, usually 3-6 hours. The Bylin et al., (1985) study suggested some effects were noted at 0.1 ppm. However, it is in contradistinction to most all other reported studies. Sandstrom (1990) recorded mild nasal irritation at 4.0 ppm for 20 minutes, and increased airway resistance was reported in healthy subjects at exposure concentrations 1.5 to 2.0 ppm (Mohsenin 1987; Frampton 1991). Others have reported no effects among healthy subjects at levels between 2.0 and 4.0 ppm (Beil and Ulmer, 1976; von Neiding, 1979; On Neiding and Wagner, 1977; Linn et al., 1985; Mohsenin et al., 1988; Sandstrom et al., 1990).

Nitrogen dioxide exposure at levels above 1.5 ppm were reported to initiate an inflammatory response and alter numbers and types of inflammatory cells in the distal airways or alveoli. However, these responses depended upon higher exposure concentration, extended duration, and greater frequency of exposure. Nitrogen dioxide may alter pulmonary cell function and cellular production of mediators in some receptors, and these factors may be important in the receptor's lung host defense responses.

The data in asthmatics also seems to be somewhat contradictory, and in the final analysis, the severity of the pre-existing asthma may be the deciding factor in any response. Airway responsiveness in asthmatics occur at levels of 0.3-0.5 ppm, yet changes in lung function have not been identified even at exposures up to 4.0 ppm. Von Neiding and Wagner (1977; 1979) found no effects in patients with COPD from exposures below 2.0 ppm for short duration, while Bauer (1987), and Morrow and Utell (1989), saw responses with 0.3 ppm for 4 hours.

In asthmatic subjects, increased airway responsiveness has been seen after NO<sub>2</sub> exposure when subjects were challenged with a variety of mediators, including cold dry air, cholinergic and histaminergic chemicals, and SO<sub>2</sub>. The challenges occur prior to or after exposure. Although levels as low as 0.3 ppm may have been a stimulus, the presence of these responses appears to be influenced by exposure protocol, particularly whether or not the exposure includes exercise, and the individual's susceptibility not only to NO<sub>2</sub> but also to the challenge agent. The ventilation rate increases with exercise and alters the depth of inhalation as well as the amount of material inhaled. The bioavailable dose can change quite significantly. Modest changes in spirometric measures of lung function (3 to 8%) were reported in some asthmatics and COPD patients under certain exposure conditions. However, it is questionable since the ailing patient may have been sensitized either by

previous exposure, or will respond to the existing physiological deficit. At any rate, the data is equivocal, since on various occasions it is, or is not seen at all in some or none of the exposed subjects. No clear association between lung function responses and respiratory symptom responses and responders can be identified.

There is little supportive evidence of a definitive concentration-response relationship of changes in lung function, airway responsiveness, or cellular mediated symptoms that are related to the NO<sub>2</sub> levels and duration of exposure. Other commonly occurring NO<sub>x</sub> species do not appear to cause any pulmonary function responses at concentrations generated in the ambient environment, even at higher levels than in worst-case scenarios. However, not all nitrogen oxides acid species have been studied. Perhaps most importantly, existing levels of indoor and outdoor nitrogen species may play a significant role in sensitizing exposed subjects, predisposing these receptors to effects that may be greater than expected. Accordingly, the data is suggestive but difficult to interpret with any degree of certainty.

Available experimental and clinically controlled data of exposures to NO<sub>2</sub> suggests an exposure of 4.0 ppm of NO<sub>2</sub> for one (1) hour or less, appears unlikely to be able to cause a significant adverse effect or irreversible harm or organ dysfunction in a healthy normal adult (Hazucha, 1995; Jorres and Magnussen, 1990; Linn, 1985;; Frampton, 1991), nor in some asthmatics (Linn and Hackney, 1984).

There does appear to be a very small group of ultrasensitive receptors and they may be exceptional responders. Subjects with pulmonary disease, such as asthma, chronic obstructive pulmonary disease or emphysema, and those aged and young children susceptible to the adverse physiological effects of exposure, may experience significant changes in reduction of airway responsiveness at levels at or above 1.5 ppm if exposed for longer than one hour. Due to uncertainty in the experimental data, it is recommended these individuals should not be unnecessarily exposed to NO<sub>2</sub>, above that level.

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## **5.0 TOXICOPATHOLOGY OF NITRIC ACID (HNO<sub>3</sub>) EXPOSURE**

There are very few available references in the database that provide well developed, or accurate data of dose response or the clear cut toxic effects of low level short term exposures to nitric acid. Those references that do provide relevant information of any effects and dose are cited, together with other factors considered of importance.

### **5.1 ANIMAL STUDIES**

#### **Effects of Exposure To Nitric Acid Among Animals.**

In an effort to determine the possible toxicity of nitric acid exposures, several reports of the pulmonary effects of HNO<sub>3</sub> exposure were generated in the early 1980's. Obliterative bronchiolitis, acute bronchitis, bronchiolectasis, bronchiectasis and biochemical changes in collagen and elastin have been identified (Coalson et al., 1988; Christensen et al., 1985; Abraham et al., 1982).

Abraham used groups of seven, normal and allergic sheep, exposing them to 1.6 ppm HNO<sub>3</sub> vapor for four hours via a nebulizer. The exposed animal groups failed to demonstrate bronchoconstrictive effects although 24 hours after exposure, a challenge by carbachol indicated a mild increase in hyperresponsiveness. Exposure levels were measured by Drager Detector Tubes, and recorded as 1.6 ppm +/- 10%. Although such measurements are acceptable for field measurements of exposure, they are insufficient when analyzing exposures for clinical correlation of physiological changes.

Coalson (1985) instilled 0.5% nitric acid in 0.5 ml saline per 100 gm body weight into the orotracheal airways of Syrian Golden Hamsters and characterized various physiological changes in lung tissue. They found numerous mild to severe cases of acute bronchitis, acute or obliterative bronchiolitis, bronchiolectasia and bronchiectasis as well as changes in collagen and elastin as a result of nitric acid exposure. However, the acute toxicity became evident when several animals died before day three of the exposure. Upon analysis of tissue, all the dead animals demonstrated evidence of severe hemorrhagic pulmonary edema. Among the animals alive at three days post exposure, there were striking losses in bronchial epithelial tissue as well as alveolar hemorrhage and edema. The acid caused severe irritation and hydrolysis of bronchial and alveolar tissue. Christensen (1985) also used a technique of instillation of HNO<sub>3</sub> in mice and in addition to the tissue changes noted

above, identified secretory cell metaplasia that persisted for 17 weeks after exposure and may contribute to chronic obstructive pulmonary disease.

## 5.2. HUMAN EFFECTS

### Physical-Chemical Factors In HNO<sub>3</sub> Toxicity In Humans

Nitric acid appears as a potent respiratory irritant in animals. However, early work by Sackner and Ford(1981), cited by Hackney (1985), shows that human exposure to 1.6 ppm HNO<sub>3</sub> vapor failed to elicit any changes in pulmonary function. However, some other physical form could cause adverse effects, for example nitrates could elicit an adverse effect. Several investigators cited by Hackney showed that no effects could be identified even under exposure to sodium or ammonium nitrates.

Reports that physical and atmospheric conditions may modify exposure conditions and their potential for adverse effects have also have been shown to be without serious merit. The particle aerodynamic size and relative humidity, especially fog, were examined on effect of particle size and acid deposition within the human airways (Hackney et al., 1985; Larson, 1989; Bowes et al., 1989; and Balmes et al., 1989). However these are without effects.

In studies on gas/liquid partitioning of acid species, soluble species such as HNO<sub>3</sub> are found mainly in fog or cloud droplets, but not in acid haze particles (pH <2), (Larson, 1989). It was proposed that water droplets in fog, may concentrate soluble air toxicants and when inhaled deposit preferentially at the airway bifurcation, leading to a higher concentration. However, it has been shown that fog droplets are about 10 µm in size, and too large for deposition in the lower respiratory tract when examined in human volunteers (Bowes et al., 1989). Effectively, that would reduce the concentration reaching the lower lung and reduce the potential for dose. In addition, ammonia, naturally produced in the airways, should neutralize much of the inhaled acid. "Occupational experience and controlled exposure studies indicate that irritation by aerosols is most likely at concentrations too high to occur in ambient air" (Hackney et al, 1989).

However, the one factor that does effect the potential for inhalation into the lower reaches of the lung, and may be causative factor in bronchoconstriction, is titratable acidity (Balmes et al., 1989; Fine 1987). In these studies, humans were exposed to three minute hyperosmolar fogs containing H<sub>2</sub>SO<sub>4</sub>, or HNO<sub>3</sub>, or both acids, or to HCl and examined for

airways effects. The results show exposure to the acidity causes an increase in airways bronchoconstriction. Each of the exposures produced an equivalent bronchiolar constricting potentiation effect and thus indicates that chemical species or composition is not the important factor. These results indicate titratable acidity as the major variable affecting airways hypereactivity. However, it is important to note that hyperosmolar fogs generated in the laboratory, had unnaturally high levels of  $H_2SO_4$  and 6 to 7 times the amount of water that could be found even in the most severe natural environmental fogs.

### **Clinically Relevant Nitric Acid ( $HNO_3$ ) Exposures**

The effects of  $HNO_3$  vapor exposure (in vivo) have been examined in two recent studies. Becker et al. (1992) exposed nine healthy subjects to  $200+/- 4g/m_3$  (80 ppb) of  $HNO_3$  vapor for 120 min, including 100 min of moderate exercise (VE 42 L/min). Bronchioalveolar lavage performed 18 h post-exposure indicated increased phagocytic activity of macrophages harvested from the  $HNO_3$  exposed lung. Alveolar macrophages also showed increased resistance to infection with respiratory syncytial virus. Compared to air exposure, there were no increases in inflammatory mediators (such as prostaglandin E2, leukotriene B4, C3a, or neutrophils) or in cell damage indicators such as lactate dehydrogenase (LDH) or proteins from bronchioalveolar lavage fluid. The absence of markers of tissue damage (LDH) or permeability (lavage fluid protein), suggest that, under these exposure conditions,  $HNO_3$  did not cause frank tissue damage.

Aris et al. (1991a) exposed 10 healthy subjects to  $0.500 mg/m^3$   $HNO_3$  vapor for four hours, including moderate exercise. Lavage fluid was obtained from both bronchial as well as bronchioalveolar washings, and bronchial biopsy specimens were obtained. No change in LDH levels or lavage protein were observed as a result of  $HNO_3$  exposure. These investigators found no differences in differential cell counts in the lavage fluid of both bronchial and bronchioalveolar washings. They also exposed a different group of subjects to  $0.500 mg/m^3$   $HNO_3$  plus  $0.20 ppm O_3$  and found no potentiation of the  $O_3$  induced inflammatory response by the addition of  $HNO_3$  vapor to the exposure. Their data suggest that, at these concentrations,  $HNO_3$  does not cause tissue injury, nor does  $HNO_3$  alter the inflammatory response typical of  $O_3$  exposure.

The acute and or delayed toxicity of  $\text{HNO}_3$  may be generated by nitrogen species other than nitric acid, (nitrogen dioxide and/or nitrous acid), (Hejela 1990) or by those of concomitant exposures, such as Ozone, (Aris et al., 1993) or from nitrous acid (Beckett et al., 1994). Hejela (1990) reports a case of fatal pulmonary edema in three young healthy men, pulp mill workers, exposed to nitric acid fumes when a tank with 1736 Liters of 68% nitric acid exploded. They were cut off from escape route and acutely exposed to fumes during the fifteen minutes it took for the men to escape the building.. After exposure, there was a period of supposed well being lasting a few hours followed by a rapidly progressive non-cardiogenic pulmonary edema. All three men died from rapidly progressive pulmonary edema that resulted from increased permeability in the pulmonary microvasculature. The response to capillary injury appeared to be the result of a combination of nitrogen dioxide, nitric and nitrous acids. The clinical picture among all three indicated very high blood pressure and high levels of segmented leukocytes. Pathological analysis showed bronchiolar necrosis, capillary engorgement and alveolar edema. The lungs were filled with a protein rich, frothy fluid (serum albumin, fibrinogen, IgM and IgG) that flowed out from the mouth and nose prior to death; this suggests very high capillary permeability (Hejela et al., 1990).

Aris et al., (1993) studied the effects of air or nitric acid exposure ( $0.500 \text{ mg/m}^3$ ), or of ozone versus ozone and nitric acid exposures on 10 healthy athletes, during moderate exercise for four hours. The results of examination failed to demonstrate any differences in bronchiolar lavaged cells, in biochemical constituents between the  $\text{HNO}_3$  and air exposures, nor between the nitric acid plus ozone versus the ozone alone exposures. Their data supported the conclusion that  $\text{HNO}_3$  does not cause either proximal nor distal lung airway injury, nor does it potentiate the inflammatory response in healthy humans.

Beckett et al., (1994) examined the effects of 0.65 ppm of nitrous acid ( $\text{HNO}_2$  ) on 11 adult asthmatics who were exposed for three h and performed moderate exercise for three 20 min. periods. A significant decrease in forced vital capacity was seen after 25 min and 85 min. post exposure. The level and duration of exposure, indicates that lung mechanics are slightly altered, and produces mild symptoms of irritation in asthmatics but does not induce significant airflow obstruction. This level of exposure was a weak sensory stimulus. The change in FVC and absence of any effects on Forced Expiratory Volume (FEV) or

Maximal Mid Expiratory Flow (MMEF) indicate this level of exposure may be slightly above the threshold that can alter lung mechanics.

### **Effects of Nitric Acid Vapor on Asthmatics**

Koenig and associates (1988, 1989) have recently reported preliminary results of a study of adolescent asthmatics exposed to HNO<sub>3</sub> vapor. In the first report (Koenig et al., 1988), subjects were exposed to 50 and 100 ppb HNO<sub>3</sub> and to 50 ppb HNO<sub>3</sub> plus 68 µg/m<sup>3</sup> H<sub>2</sub>SO<sub>4</sub>. The average FEV1 decreased following exposure (30-minutes rest followed by 10-minutes mild exercise) under all three conditions, although there were no significant differences among the responses to these exposures.

In a repeat study of the above, Koenig et al. (1989a) reported the responses of adolescent asthmatics to a 40-minutes exposure to 50 ppb (2.0 µM/m<sup>3</sup>) HNO<sub>3</sub> vapor exposure via a mouthpiece exposure system. In this study, after 30 minutes of rest and 10 minutes of exercise while breathing HNO<sub>3</sub> vapor, there was a 4.4% decrease in FEV1 compared to 1.8% decrease after exposure to air. There was a 22.5% increase in total respiratory resistance detected after HNO<sub>3</sub> exposure, compared to a 7.5 % increase after inhaling air. In another study by Koenig et al. (1989b), subjects were exposed to air and 16 ppb HNO<sub>3</sub> twice: once without and once with a preliminary gargle of lemonade which was intended to reduce levels of oral ammonia (NH<sub>3</sub>). During the 45-min exposure, subjects exercised twice for 15 min (ventilation rate of 25 L/min.). Baseline oral NH<sub>3</sub> of 318 + 84 ppb was reduced to 113 + 98 ppb after lemonade gargle. There were small, but statistically non-significant decreases in FEV1 after the exposure. The decrease was 3.3% after HNO<sub>3</sub> alone and, -1.7% after both air and HNO<sub>3</sub> plus lemonade. Similar trends (9.4%, HNO<sub>3</sub>; 5.5%, HNO<sub>3</sub> plus lemonade; 5.1%, air) were detected for fixed vital volume. The data did not support the hypothesis that reduction of oral ammonia using a lemonade gargle would increase the response to HNO<sub>3</sub> because HNO<sub>3</sub>, in the absence of ammonia, would not be converted to ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) in the upper airway. Nevertheless, the authors suggest that, in mixtures of HNO<sub>3</sub> vapor and H<sub>2</sub>SO<sub>4</sub> aerosol, gaseous ammonia (NH<sub>3</sub>) may react more rapidly with the gaseous HNO<sub>3</sub> than with the aerosol, reducing the potential for the neutralization of sulfuric acid. However, this is speculation dependent upon known

physicochemical properties of  $\text{HNO}_3$  vapor and  $\text{H}_2\text{SO}_4$  aerosol; it is not supported by evidence of experimental observations.

### **5.3 CONCLUSION**

Nitric acid aerosols demonstrate the typical toxic response characteristic of acid irritation, even at low doses. Exposure to nitric acid levels in the range of 50 to 200 ppb for short periods of time may cause an adverse effect or response in pulmonary function among sensitive receptors but it does not appear likely in healthy adults. Acute exposures to very high doses have caused fatalities from severe pulmonary edema.

The results of the literature review indicate the toxicological database is deficient and lacking in information suitable for preparing conclusions of acceptable exposures. The database of information is also inadequate for the preparation of any conclusions relative to the possible adverse health effects that may occur from short term low level exposures.

## **6.0 PULMONARY TOXICOPATHOLOGY AND ISSUES FOR FURTHER RESEARCH**

It has been shown that humans can be adversely affected by airborne exposure to chemicals that interact with respiratory tract tissue and will alter airway mechanics or gas transfer. A physiological response to any exposure that adversely affects airflow and/or transfer of pulmonary gases, affects the function of the pulmonary system and it is thus considered a significant adverse response; it is also possible that a significant adverse response, to some defined level of exposure and duration, may progress to an irreversible pathological state, and ultimately alter organ function. Lung cancer, bronchitis, asthma, edema, emphysema, extrinsic allergic alveolitis, chronic obstructive pulmonary disease (COPD), adult respiratory syndrome, (ARDS), and effects on the microvasculature, the epithelium or parenchyma is considered a very significant adverse response. However, these conditions have a greater potential to occur when exposures are exceptional such as when very high concentrations or chronic exposures occur. Under the conditions of acute very short term exposures to low doses, detecting an adverse effect is difficult at best, and makes accurate projections of allowable exposures a difficult undertaking. Current philosophy suggests that under the conditions of very low dose-short term occasional transient exposures under consideration, serious or irreversible harm is entirely possible but hardly likely. Exposures to these chemical species appear unlikely to initiate any significant sequelae, unless there is a susceptibility or idiosyncratic response.

### **6.1 Pulmonary Toxicity: Pathology Or Adaptation.**

A major dilemma in the determination of physiological endpoints, established by using histopathological techniques is, the difficulty of clearly separating an adaptive response, a mild transient effect, an adverse effect that may also be transient, an acute toxic response that may be an irreversible effect and those which initiate chronic disease. An end point that is observed and described by the investigator(s) as a toxic response associated with exposure may be a toxic response or it may be a "physiological adaptation" that occurs within the flexible framework of the mammalian physiology.

Respiratory tract lesions or changes in tissue may be reported as an adverse or toxic effect, however, it may simply reflect physiological adaptation to noxious stimuli. An

example is squamous metaplasia (SQM) which has frequently been reported as an adverse response. Is this change an acceptable adverse response end point. SQM is replacement of one adult cell type with another adult cell type, within the epithelium of a tissue or organ, due to a stimulus. It is an adaptive, reversible response that occurs from persistent insult that is better able to withstand the adverse environment created by the insult ( Robbins et al., 1994). The most common adaptive metaplasia occurs in the human respiratory epithelium, usually as a response to chronic irritation from tobacco smoke, Vitamin A deficiency or a noxious stimulus. The situation can be reversed by removing the stimulus.

Stones in the salivary gland, pancreatic or bile ducts may initiate a metaplastic change. Stratified Squamous epithelium is rugged, and survives under most circumstances where special epithelial tissue does not. The organism responds to a stimulus by raising a defense barrier. Can that be interpreted as a toxic end point?

Squamous metaplasia in laboratory animals may result from other factors; rodents being housed on soiled bedding where generated NH<sub>3</sub> is a stimulus. Where Vitamin A deficiency, or a viral infection exists, or perhaps it is the result of age dependent anatomic variation which has been shown in controls where 0-10% of the control animals respond with SQM without benefit of chemical dosing. The response may not be adverse, especially if the insult is an acute, short term exposure, (as for a one hour rocket emission) with an acute, potentially reversible response.

However, if the insult is of extended duration, and of a severity level to cause a loss of mucus secreting ability with the change, then the tissue change is undesirable and may predispose to chronic irreversible condition and possible malignancy. However, if the change is transient, short term and reversible, without loss of ability to secrete mucus, it may not be an adverse toxic response but merely reflect the change within the wide spectrum of physiological boundaries.

The analyst, objectively believing a change occurred, may report the adaptive response as a toxic response. This outcome will create a significant bias toward risk characterization and development of acceptable levels of exposure. Thus an acceptable structure for classifying adverse effects, as opposed to a physiological adaptive change, is required to determine when does toxicity begin and what pathological sequelae may develop from that exposure level. This program of classification would provide the needed

support for structuring a standard interpretation of pathological processes and mechanisms of adverse effects.

Appearance and accumulation of macrophages and associated inflammatory cells, changes in cell superstructure, in keratin, or the distribution of mucin cells, oxidation of epithelial molecular side chains and terminal residues, and a host of other situations may also reflect similar adaptations.

## **6.2 Inflammatory Response: Transient Versus Significant Response.**

Most of the available data cited measurements of any change in pulmonary function and the presence of inflammatory response cells present in bronchioalveolar washings. These changes are not necessarily adverse. The pulmonary tissue is covered with a protective mucous layer to defend against irritants and toxic insults, such as HCl and NO<sub>2</sub>. An airborne exposure that penetrates the protective mucous layer of the lung, can irritate the tissue and may initiate an inflammatory response. The effects of exposure may initiate a mild, transient cell surface irritation that resolves in a day or two. If the irritant exposure concentration is increased, it may solubilize in the mucous layer, damaging underlying cells and tissue. This will initiate the inflammatory response, a host defense and damage repair mechanism. It is an adaptive physiological response designed to remove the irritant and repair any tissue damage. During this process, soluble products cause the influx of leukocytes to mobilize to the site of insult, and injured cell materials or physical irritants initiating the response, are removed by cellular digestive enzymes and injured tissue repair process then proceed. However, a serious potential for adverse tissue effects occurs when phagocytic products, released from granulocytes, macrophages or other cells to digest the irritant, spill out into the surrounding tissue, and persists in digestive activities, damaging normal tissue. This persistence can initiate further tissue damage at the injury site and produce adverse effects. Lysosomal enzymes, oxygen derived metabolites and the products of arachidonic acid metabolism, initiate leukocyte dependent tissue injury that may result in additional pathology. Thus the appearance of the inflammatory cells and their products may be considered a critical juncture at which significant adverse effects may potentially occur. The inflammatory process has been well studied and much is known, but it is not completely characterized. None the less, it is well known that this process can initiate emphysema, rheumatoid arthritis, and chronic pulmonary disease, especially when

chronic irritation causes the inflammatory response to persist and exaggerates the response and those activities. These effects provide a significant level of consideration in interpretation of adverse effects and preparing estimates of allowable exposures and duration.

### **6.3 Acid Aerosol/Gas Exposure and Human Health.**

In assessing the available data, several issues of concern have become apparent and the database has serious gaps that need to be filled. Presently there is an equivocal and inconsistent body of experimental and clinical evidence of the actual effects of exposure to these emission products. Concurrently, there are no data that could provide effective support for predicting responses from the short term exposures that may be expected from rocket emissions. Thus, additional toxicological testing will be required to determine the possible equivalent human response to the exposure when it is delivered over the short time periods of one hour or less.

Generally, healthy humans do not appear to be affected to most low level exposures. However, some asthmatics, and subjects with chronic pulmonary disease or lung dysfunction, appear to be affected by some exposures. The source of consternation is that positive responses are reported at the low concentrations of exposure yet negative responses are reported from exposure to higher exposures. The responses tend to be less than significant and demonstrate a wide variation in responses, painting a confusing picture.

The role of exposure to these inorganic emission species in effecting an adverse response among various human receptors requires further additional investigation and analysis. Although the report examined three different chemical species, the same questions need to be asked for each of the emission products as well as the effects that can be generated from multiple exposures, since their effect may impact the same pulmonary tissues. They may also affect different tissue or regions within the respiratory tract. The data examined are suggestive but lack consistency.

Nitrogen oxides, hydrochloric and nitric acids (NOx, HCl, HNO3) impact the respiratory tract and affect different pulmonary region tissues differently. The toxic responses and the variations in these responses need to be clearly identified. In order to provide effective answers to the question of toxicity and receptor susceptibility, and possible multiple chemical exposures, the issues listed below, applicable to all the species of concern (NO2,

HNO<sub>3</sub>, and HCl), will require additional research to determine target organ(s), tissue and cells of impact, their toxic mechanisms and corresponding levels of exposure.

#### **6.4 Interaction Of Exposure Estimates On Risks From Toxic Response**

The data base is not entirely adequate, but does allow the reasonable scientist to discern that adverse effects can occur from exposure to these several gas/aerosols. Analysis of these data indicate that the response from exposure to acid gases does not follow linearly with Haber's Law, when either duration is extended or concentration is high. An exposure of less than thirty minutes will yield different responses than predicted by the same integrated dose when the exposure is of a long duration. Thus the alternative is to understand the statistical variations and probability of some adverse response occurring given the diversity of dose, duration, receptor location and sensitivity. Such parameters are critical to preparing estimates of risk and ultimately to risk management. Given the broad ranges and varying combination of exposure parameters, Monte Carlo analysis is thus recommended for use in preparing mean and reasonable maximum estimates of exposures under varying duration of exposures from normal or catastrophic scenarios. The knowledge derived from analyses of exposure concentrations and duration of exposures, will provide a suitable and useful range of exposure concentrations. That information of possible exposures and human toxicological response will provide for effective risk communication and range decision making.

#### **6.5 RECOMMENDATIONS FOR FURTHER RESEARCH:**

- Can infrequent exposures ( low ppm, short term < 1 hr.) to these chemical species of concern cause consistent and reproducible long term changes in lung function (i.e., increase in respiratory symptoms, airways hyper-responsiveness, chronic inflammatory response, changes in inflammatory cells and soluble mediators) in healthy subjects during the short term, low levels of exposure expected from rocket emissions?
- What is the probability that an acute exposure to the combination of emissions will contribute to the chronic effects of lung function, promote development of, or aggravate an existing respiratory disease, or cause acceleration of the age-related decline in lung function?

- Is there a laboratory animal or mathematical model that can provide a dose response curve that will accurately gauge the effects occurring at varying dose levels?
- Are sensitive groups such as children, infants, toddlers, or the aged and infirm, or patients with asthma, chronic obstructive lung disease, emphysema or some other lung disease at special risk for adverse health effects. Can the these sensitive human groups be identified without exposure to these species of concern?
- What role does each species play in affecting the cellular response and soluble mediators during an inflammatory response among healthy individuals or subjects with lung disease? Specifically, does exposure cause; microvascular injury and capillary permeability, altered mucociliary clearance, increase in local blood flow, changes in the influx of inflammatory cells or proteins (inflammatory response), edema and cell damage. Are the inflammatory cells such as alveolar macrophages, mast cells, neutrophils (polys) and/or eosinophils flowing into the interstitium and airways, causing the damage or are the secretions and enzymes of inflammatory mediators, or epithelial desquamation the responsible factors?
- How does the presence of the three species affect potential toxicity; is it synergistic, antagonistic or additive? Whom do they affect and how?
- Addition of buffers to exposures of acid gases and aerosols tends to increase toxicity of acid aerosols. Is the neutralizing effect of exhaled ammonia (NH3) produced by humans during controlled exposures, affecting potential toxicity and response?
- Evidence for response from concurrent exposures suggests significant synergistic effects not seen when exposures are sequential? What are the potential effects of combined exposures to these species in the presence of ambient levels of ozone?
- Does exposure of these species increase the potential for increased airway responsiveness, altered lung function, or to challenge by bronchoconstrictors, cold-dry air, exercise or specific antigens?
- What is the time course of a response to acute exposures? Are responses immediate or delayed or are there both? Do these responses increase or decrease with extended duration of exposure?
- How is the time course of response affected by repeated NO<sub>2</sub> exposures? Do responses increase or decrease with increased frequency of exposure?

- Does NO<sub>2</sub> also increase airways responsiveness when humans are simultaneously exposed to other pollutants, acid aerosols, or aluminum particulate.
- Does NO<sub>2</sub> or the acid aerosol exposures alter respiratory tract immune response and host defenses, or epithelial permeability or local or systemic immune response to infection? As a consequence, does exposure to one or more species, impact host defense system components and modify response capability?
- Are phagocytic mechanisms and production of oxygen killing species for removal of microorganisms impaired by such exposures?
- Is the inflammatory response or tissue injury caused by microorganism infections worsened by coincident exposure to any of these three species?
- A major problem in assessing the database is the lack of a classification scheme that can be used for identifying toxic or pathological responses. When, or at what point does a toxic response occur as opposed to a physiological adaptive response, which is reversible or is not a chronic effect? How do we truly identify receptors as sensitive and at greater risk?
- Have background levels of nitrogen oxide species caused certain susceptible individuals to become hyper responsive when challenged by other bronchoconstrictors?

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## **7.0 - USE OF AN ORDINAL REGRESSION MODEL FOR ESTIMATING SHORT TERM EXPOSURE RESPONSE FUNCTION.**

The purpose of this section is to recommend an analytical approach useful for estimating the probability of response for a specified severity of effect associated with an exposure of short duration. This estimate is described as the Exposure Response Function (ERF), used in the LATRA/REEDM risk model to assist launch commanders in determining the public health risks of rocket motor fuel combustion emission exposures. The resulting information supports Go/No Go decision making for a rocket launch.

### **7.1 BACKGROUND**

The standard methodologies used for preparing acceptable risks and promulgated by various regulatory agencies were reviewed and evaluated in order to determine their applicability to the emission exposures from rocket fuel combustion. Generally, risk assessment methodologies, used for non-cancer end points, focus on chronic or lifetime exposures using a single point reference dose, called the RfC. The RfC is the proposed inhalation reference concentration that, when inhaled over a lifetime would not be likely to cause any serious or significant adverse health effects. The RfC is derived from the No Observed Adverse Effect Level or the Lowest Observed Adverse Effect Levels (NOAEL, LOAEL), determined from experimental laboratory animal dose response data. The NOAEL or LOAEL value is adjusted to a human equivalent dose, using ratios of human and animal physiological parameters. Further adjustments are made using a series of uncertainty factors, that account for chronic and/or acute doses, inter- and intra-species variability and uncertainty, to derive an acceptable exposure level that offers protection to human receptors. The resulting value is the RfC, which is considered a level that is without appreciable risk, even during a lifetime of exposure.

However, most such methods of risk estimation suffer some degree of credibility for several reasons. They use human judgments, derived data, fixed lifetime exposures, assumptions that the exposure concentration is a constant for the duration of exposure and use of data without consideration of mechanistic and species specific determinants

(Jarabek, 1995). An assessment of a non-cancer health risk is highly dependent upon exposure concentration and duration and ideally the assessment of a short term risk should reflect response information across the range of exposure duration as opposed to a single point. Again, the standard methodologies neither use all the dose data nor do they use the data developed for any particular duration of exposure.

Recently, several papers were published that consider these parameters and integrate these data into a short duration exposure risk estimate using a mathematical logistic regression model (Guth et al., 1991; EPA, 1991; Guth 1996). The mathematical logistic regression model is used in statistical analysis to find the best fit, most closely related, yet biologically reasonable model, that describes the relationship between an outcome (response), and a set of independent variables (concentration and duration). For rocket emission exposures the LATRA/REEDM models describe the exposure level at various points of the toxic corridor and estimate the risk. Given the appropriate kinds of data, the logistic regression model can then provide the exposure reference levels that can be used for estimating risk.

The short term logistic regression model (STLRM) has been used for estimating the risk for short term exposure response, and provided there is a suitable type and amount of data, is adaptable to degrees of severity of effects. The logistic model appears to be useful since it considers duration of exposure, exposure concentration and can merge available differences of discrete classes of severity of a biological response. The STLRM results in a discrete characterization of a category of effect, minimizes subjective input that might accompany such an analysis, provides for possible differences in exposure responses and increases the level of confidence in resulting estimates. It is therefore proposed for estimating the health risks from acute exposures to rocket fuel combustion emissions. The process is simple, yet effective and can serve until a more useful model is identified.

## 7.2 SHORT TERM LOGISTIC REGRESSION MODEL (STLRM)

The proposed use of a logistic regression model that refers to the simple form of the model and assumes the data being modeled comes from a homogeneous data base is proposed as a useful approach. The general form of the multiple log regression model is written as,

$$\Pr(Y \geq S | C, D) = H(\alpha + \beta_1 \ln C + \beta_2 \ln D),$$

where C = concentration, D = duration, H is a functional relationship, and S = class of severity of response (=1,2,3...N) and Y is the ordinal severity category for a particular group. The statement then reads, the probability (Pr) of equaling or exceeding some given severity category (S) at a specified exposure level (C) and duration (D) is a function of the natural log of the concentration and log of the duration; the  $\alpha$  and  $\beta$  coefficients are estimated model parameters. This formula lends itself to non-cancer risk assessment because if S = the NOAEL (or AEL) then, given a fixed exposure concentration and duration of exposure, the model can estimate the probability of exceeding the NOAEL (or AEL). At a particular concentration and duration of exposure, the model estimates the probability of observing an effect, greater than the NOAEL (or the AEL, whatever the case may be). The model then becomes,

$$\ln(P/1-P) = \alpha_s + \beta_1 \ln C + \beta_2 \ln D, \text{ where,}$$

P = probability, s = severity level, C = concentration, D = duration, Ln = natural log.

This model was used to statistically analyze the animal and human toxicity data extracted from the experimental studies for  $\text{NO}_2$ . This model appears to lend itself to identifying the exposure response function needed for LATRA since the duration of exposure to a rocket emission is assumed or generally considered to be one hour or less and emission concentration within the toxic corridor is estimated via the REEDM model. Given those values, the probability of the severity of an adverse effect may be calculated and the result provides the decision maker with support to justify the GO/NO GO launch command based upon the probability of an adverse effect. The logistics regression model was applied to the  $\text{NO}_2$  data only since other authors have analyzed the HCl data (EPA, '91).

### **7.3 RESPONSE LEVELS**

The proposed regression analysis model uses quantal response data with concentration and duration data and establishes classes or categories of effects. These classes of severity of effects describe the different biological effects that are associated with different exposures. The classes will be suggested by the data, is flexible and the numbers of classes can be varied. A major difficulty comes in the variations in individual interpretation of what constitutes mild, moderate, serious, and severe, or statistically or biologically significant, and thus becomes a source of discord. In these situations, an expert consensus will normally be required for making those judgments.

The impact of various definitions and interpretations of biological effects is minimized when few classes are defined. In the case of the NO<sub>2</sub>, the scale was based upon the authors reported adverse effect on animals and/or humans in the cited experimental studies. In these studies, the authors cite a single target organ affected using measured responses to inhaled NO<sub>2</sub> exposures, the respiratory tract.

Any subject included in the AEL severity of effect category, was placed there according to a conclusion by the study author(s). The AEL describes any measure demonstrating or effecting a physiological change in the respiratory tract cells, tissue or function as an end point, and concluded by the author to be a biologically or statistically significant effect. Change in airway resistance, response to bronchoconstriction challenge, numbers and/or types of cell populations or enzymes present in bronchioalveolar lavage are all included as adverse (AEL) responses. No deaths were recorded or described so that no other severity level is described in the human studies. These categories are not identical to, but are consistent with those concepts proposed by Guth (1991, 1996).

The effects measured refer to several end points and include, pulmonary volume, air way responsiveness and cell types, numbers or enzymes present in bronchioalveolar lavage fluid exams. In all the cited studies, NO<sub>2</sub> exposure impacted the respiratory tract epithelial tissue to initiate some response and affect some functional change. Thus any change within the respiratory tract, described as biologically or statistically significant by

the author, is considered a recognizable departure from the norm and is considered an adverse effect level (AEL). If the exposure fails to produce a recognizable change of the normal or baseline values measured, it is a No Observed Adverse Effect Level (NOAEL). One additional class of response, found primarily among the high level animal exposures is the frank or fatal effect level (FEL). The end point in these studies was mortality. Thus for this analysis, the classes of end points are the NO Adverse Effect Level, (NOAEL) or the Adverse Effect Level (AEL), for the human studies. In animals mortality studies there were three classes, NOAEL, AEL or FEL.

#### **7.4 TEST SUBJECTS**

The types of human subjects included in the cited studies were asthmatics as well as non-asthmatics, young and old, athletes and non-athletes and they represent, albeit not ideally, a reasonably typical group of expected human receptors. Biological data analysis indicates the asthmatics in the group did not respond in an exceptional manner, nor were they different than the non-asthmatics. In none of these studies did the exposed asthmatics suffer any acute attacks or episodes of respiratory distress, typically seen among asthmatics. Additionally, the adverse effect level can be classified as a single entity since the only tissue affected is the respiratory tract. Only one severity category, the AEL, was used since the epithelial tissue of the entire respiratory tract is considered as a singular target. Except for the vocal chords, the entire respiratory tree including larynx, trachea and bronchioles, is pseudostratified, tall columnar ciliated epithelial tissue. Thus, airway hyperresponsiveness, initiation of pulmonary volume changes, the influx of inflammatory cells and/or enzymes in the bronchioalveolar lavage fluid refer to responses initiated by NO<sub>2</sub> stimulating the epithelial tissue surface receptors. The changes in respiratory tract tissue responses suggest mild sensory irritation and early stages of the inflammatory response. Were the concentrations severe, evidence of the chronic inflammatory response would be observed and they would signal a change to a serious effect. Evidence of the chronic inflammatory response is indicated by an increase in highly protein rich exudate filling interstitial spaces (edema), destruction of type I cells and replacement by type II cell types and fibrosis. Such changes were not described or observed, thus the end point(s) described

appear to indicate various transitional stages and degrees of an acute inflammatory response. Collectively, these effects are classified as a low level adverse response level.

## **7.5 ANIMAL EXPOSURE STUDIES**

Previously cited animal studies describing the effects of acute exposures to nitrogen dioxide were reviewed and evaluated for applicability to analysis by the model. Only those studies that defined the exposure level(s), the adverse response to the respiratory tract and the number of subjects that responded adversely were included in the database for treatment by statistical analysis. The exposure concentration (dose in ppm) and duration of animal exposures to nitrogen dioxide used were extracted from the studies and compiled in an Excel database. The database constructed for this analysis included species, the exposure concentration, the duration of exposure and the effect level, as described by the author, and the citation. The only target organ of concern among all these studies was the respiratory tract. There are three severity of effect level categories for the animals, and these conform to the reported effects described by the authors in the citations. They are designated the No Observed Adverse Effect Level (NOAEL), the Adverse Effect Level (AEL), indicating some recognized effect or change occurred that moved that parameter away from the norm, and the Frank Effect Level (FEL), which indicated an animal fatality.

The cited authors' conclusion of adverse respiratory tract effects, was the only criterion used for classifying the animal response or the respective severity levels. The authors' description of a biologically or statistically significant effect, or the numbers of animals affected, were used to classify groups within an effect level. In a pathological or toxicological assessment or evaluation, statistical significance typically indicates that the response was three standard deviations from the mean and is accepted as outside the range of normal response data. Therefore if an effect, that differed from baseline norms either biologically or statistically, was noted by the authors, it was considered an adverse effect level. In the case of animals that died from the exposure, they were included in the FEL. The remaining animals were included in the categories of severity of response as reported by the author. It is important to note that a no effect determination

in a mortality study may not have been considered a no effect level in another category study.

Ordinarily, data is aggregated into subsets; that is by sex, end point or species and then analyzed. However, among the studies reviewed, it was decided to analyze the group of animal data as a single entity. Unlike the human data, these animals were consistently exposed to very high concentrations of NO<sub>2</sub>, and animal mortality was the frank effect level (FEL) end point described by the author. Based upon the earlier review of the cited references, it appears that all laboratory animals were treated according to a fairly precise and consistent set of experimental conditions. Since they were treated essentially in the same manner as all others, the FEL end point is a true reflection of the severe adverse effect of NO<sub>2</sub> exposure. Given the similarity of exposure conditions, the same target organ affected and the same response, it was concluded the data can be merged for analysis.

Table 7-1 below is a partial list of the results of statistical analyses of the animal data. The full statistical analysis is found in the Appendix.

**TABLE 7-1. STATISTICAL ANALYSIS OF ANIMAL NO<sub>2</sub> TOXICITY DATA**

TERM	COEFFICIENT	S. E.	Chi-square	D. F.	P value	Log Likelihood
Log C( $\beta_1$ )	2.037	0.248	88.40	1	0.0000	-393.7468
Log D( $\beta_2$ )	0.3696	0.131	8.19	1	0.0042	-353.6379
K ( $\alpha_s=1$ ) (NOAEL)	-9.808	1.14	Req'rd			
K ( $\alpha_s=2$ ) (NOAEL to AEL)	-10.27	1.15	Req'rd			

**Log C = log concentration**

**Log D = log duration**

**K = Intercept (coefficient from equation)**

**Req'rd = required**

The described relationship among the aggregated animal data is represented in Figure 1. Assessment of the raw data indicates the probable onset of a physiological impact

(airway resistance) to the respiratory tract in rats and guinea pigs is in the range of 2-12 ppm: this is considered a mild adverse effect level. The statistical and raw data analysis, and the resulting graph of the animal response data suggests that at 35-40 ppm there is high probability of a greater adverse effect occurring among a larger group of animals, which is probably a moderate effect. The raw animal data also indicates that at or above 50-75 ppm, a small percentage of exposed test subjects died. That group included mice, rabbits, rats and guinea pigs. Fatality among some test subjects would be considered a severe effect and would establish the level at which a frank effect level occurred (FEL). Further examination of the data confirms the severity relationship. As exposure levels increased to 100 ppm or above, a larger percentage of animals died. The Excel database work sheets with raw data and references are attached in the Appendix.

## **7.6 HUMAN EXPOSURE STUDIES**

The human studies reviewed, reported the results of low level NO<sub>2</sub> exposures among a large group of subjects. In the thirty three studies in the human database, there were 557 subjects. The studies included men and women, subjects with or without respiratory ailments, young adults, senior citizens, smokers, non-smokers and athletes. The test group of receptors in these studies tend to be a fairly broad representation of possible human receptors and may functionally represent a population that could be exposed to the emissions. Accordingly, this data may provide a good, but not necessarily an ideal, representation of the responses that may be expected from an exposed population.

In analyzing the results of the human data reported, it was found that each study directed their exposure activities to the effects on the tissue of the respiratory tract. Thus there were only two end points. The No Adverse Effect Level (NOAEL) and an Adverse Effect Level (AEL). There were no fatalities recorded among human subjects. Each end point identified, described the response or effect from NO<sub>2</sub> exposure to the respiratory tract cells. Any effect to the respiratory tract tissue, as described by the investigator(s) as biologically or statistically significant, was thus considered an AEL., and assumed no severity of effect. It was a purely a clear departure from the norms and signaled the occasion of an adverse response as observed and interpreted by the study authors. The AEL response may have been a change in tissue responsiveness, a hypersensitivity, a

response to bronchoconstrictors, or the change in the numbers and types of cells or enzymes present in bronchioalveolar lavage fluid. These responses are effectively those of the tissue impacted, which is the same throughout the respiratory tract. Since there are no fatalities in the human group, the response becomes essentially a yes (AEL) or no (NOAEL) response, and is examined as a matter of probabilities.

The ordinal regression model becomes a nominal logistic regression analysis and the probability statement becomes:

$$\ln(P/1-P) = 1.086 - 0.6684\ln C + 1.421\ln D$$

(These coefficients were developed during statistical analysis using the BMDP Statistical Software package, BMDP, V. 2, Release #7, 1992). Table 7-2 provides results of statistical analysis from the data sets reviewed.

**TABLE 7-2. STATISTICAL ANALYSIS OF HUMAN NO<sub>2</sub> DATA**

TERM	COEFFICIENT	S. E.	Chi-square	D. F.	P value	Log Likelihood
Log C ( $\beta_1$ )	-0.6684	0.113	37.42	1	0.000	-220.2208
Log D ( $\beta_2$ )	1.421	0.178	78.64	1	0.000	-240.8316
K value( $\alpha$ )	1.086	0.128	75.25	1	0.000	-239.1350
Goodness of fit	-	-	297.761	25	0.000	-

**Ln C = natural log of concentration**

**Ln D = natural log of duration**

**S. E. = Standard Error**

**K value is a constant**

## **7.7 ANALYSIS OF HUMAN DATA**

An analysis of the results of the 33 studies on human receptors tested indicates the following:

## **20 MINUTE STUDIES**

There were 5 studies, each with 8 subjects and no asthmatics. Test exposure levels were 1.5, 2.25, 3.50, 5.0 and 5.5 ppm of NO<sub>2</sub>, respectively and lasted 20 minutes.

RESULTS: all 40 subjects tested experienced an adverse effect.

## **30 MINUTE STUDIES**

There was a total of 6 studies with exposures lasting 30 minutes. Three study groups were tested at 0.30 ppm NO<sub>2</sub>. Each group consisted of 9, 13, and 15 subjects, respectively, 37 test subjects and no asthmatics. There were 21/37 positive responses (0/9, 6/13 and 15/15, respectively) and they were placed in the AEL class.

In the fourth study, 11 asthmatic subjects were tested at 0.25 ppm for 30 minutes: none (0/11) of the asthmatic test subjects were observed to have an adverse effect and all were placed in the NOAEL group.

In the fifth study group of 20 mixed test subjects (asthmatic and non-asthmatics), that were exposed to 0.18 ppm NO<sub>2</sub>, none of the test group was observed to have an adverse effect.

In the 6th and last group of 9 subjects exposed to 0.18 ppm, there were no adverse responses observed.

RESULTS: In these six studies, 77 subjects were tested, all at 0.3 ppm or less, for 30 minutes. 21 test subjects experienced adverse effects at the 0.3 ppm level. None of the responders were asthmatics. 56/77 failed to experience any adverse effects.

## **60 MINUTE STUDIES**

There were 5 studies conducted with a 60-75 minute duration exposure; and consolidated into one aggregate of the 60 minute group for analysis. Three test groups were asthmatic subjects only while two test groups were only non-asthmatics ("normals") only. Two groups of asthmatics, 10 in each group, were tested at 0.50 ppm for one hour. In one group all subjects (10/10) were observed to have an adverse response. In the second group, none of the test subjects (0/10) had been observed to have an adverse response. Since dose and duration are identical in both studies, the authors results or interpretation remain a question mark. In another group of 21 asthmatic test subjects,

tested at the slightly higher level of 0.60 ppm for a slightly longer duration (75 minutes), none of the subjects (0/21) had an adverse response.

In the fourth study of 18 non-asthmatics, a 75 minute exposure to 2.0 ppm failed to elicit an adverse response. 18/18 were placed in the NOAEL group.

A fifth study with a mixed group of 48 test subjects, with and without respiratory illness, exposure to 4.0 ppm for 75 minutes failed to elicit an adverse response.

**RESULTS:** In the three groups of asthmatics, totalling 41 test subjects, 10 subjects responded adversely. However, 31/41 asthmatic subjects failed to respond to exposures of 0.5-0.6 ppm NO<sub>2</sub>. In the two remaining groups, none of the 69 (0/69) test subjects had an adverse response. The data from the last two studies were exposures of 75 minutes and at 2.0-4.0 ppm and included asthmatics, and failed to elicit any response from among a group of 69 test subjects. Among 107 test subjects, asthmatic and non asthmatics, with a majority tested at levels above 2.0 ppm, there were only 10 positive responses, among one group of asthmatics exposed to 0.5 ppm. The remaining 97 test subjects, which included asthmatics and non-asthmatics failed to demonstrate any adverse response.

#### **EXTENDED DURATION STUDIES**

There were 6 studies with a 120 minute exposure and 8 studies that had 180 minute exposures and three with exposures of 4,5, and 8 hr. There were also three separate additional studies examined of 4, 5 or 8 hr duration. These last three were examined in search of possible exacerbating effects from exposures of extended duration.

#### **TWO HOUR EXPOSURES**

There were 183 test subjects in 6 studies;

31 asthmatic receptors only exposed @ 0.20 ppm;

59 normal receptors exposed @ 0.30 ppm,

8 normal receptors exposed @ 0.60 ppm,

59 normal receptors exposed @ 0.60 ppm,

21 female only subjects @ 0.6 ppm and,

5 male subjects only @ 1.00 ppm, respectively.

**RESULTS:** In all the above six studies, of male and female, asthmatics and non-asthmatics, exposed for 2 hours at levels of NO<sub>2</sub> from 0.2 to 1.0 ppm, there were no subjects that experienced any adverse effects from these exposures.

### **THREE HOUR EXPOSURE**

There were 8 studies using 123 test subjects with exposures lasting 3 hr.

- 1- 20 subjects exposed to 0.10 ppm; no adverse effects observed.
- 2- 34 asthmatics exposed to 0.30 ppm NO<sub>2</sub>: no adverse effects observed
- 3- 9 subjects exposed to 0.60 ppm: no adverse effects observed.
- 4- 20 subjects (mixed with asthmatics) exposed to 1.00 ppm: 8 with adverse effects, 12 with no adverse effects.
- 5- 15 subjects exposed to 1.5 ppm: 15 observed to have had an adverse effect.
- 6- 15 subjects tested at 2.00 ppm: no adverse effects observed.

**RESULTS:** Among 113 test subjects, 90 subjects did not have an observable adverse effect; 23 were observed to have had an adverse effect. Of the 23 responders, 8 were in the one group of twenty mixed subjects exposed at 1.0 ppm while 15 were in a normal group exposed at 1.5 ppm.

### **FOUR HOUR EXPOSURE:**

8 test subjects exposed @ of 3.5 ppm: 8 subjects experienced an adverse effect.

### **FIVE HOUR EXPOSURE**

14 subjects exposed @ 2.3 ppm: No adverse effects observed.

### **EIGHT HOUR EXPOSURE**

5 Mixed subjects exposed @ 0.60 ppm: No adverse effects observed.

**RESULTS :** The results of these studies suggest that an increase in the duration of exposure at levels of 3.5 ppm or higher may initiate an adverse effect on some test subjects. Levels below 2.3 ppm or less, failed to initiate observable adverse effects.

## 7.8 ANALYSIS OF ASTHMATIC RECEPTORS

The human group data was examined further to determine whether there was any unusual incidence of adverse responses or effects among "asthmatic" participants. Those are individuals considered to have some respiratory problem (they are asthmatics, bronchitis, etc.). There were 145 individuals identified with respiratory illness in eight different study test groups. All the subjects are identified as asthmatics. Among this group tested, 31 asthmatics (20%) were reported to have had an observed adverse effect; they were placed in the AEL group.

There were 145 members of the human test subjects that were identified in the study report as "asthmatics" (A) and had some form of respiratory illness. When separated into groups according to response from exposure, 31/145 asthmatic subjects had observed adverse effect from these exposures. Since 114 asthmatics failed to respond adversely, it indicates that asthmatics do not respond identically or similarly as a group, but have varying degrees of response, independent of the "asthmatic" or sensitive designation. Of the 238 mixed group normal and asthmatic (N/A) and asthmatic only (A) test subjects only 39 (16%) were observed to have had an adverse effect. Among the 557 test subjects examined in these studies, there were 455 NOAEL responses and 102 AEL responses. Of those AEL's, there were 71 non-asthmatics and 31 "asthmatics".

There were four studies reporting the effects of exposure on mixed groups of receptors. They were identified as normal/asthmatics (N/A). The authors did not identify how many asthmatics were in the study group, only that they were present within the test group. In these four mixed groups, there were 85 exposed subjects. Seventy five (88.3 %) had no adverse response and were in the NOAEL class. The eight (8) subjects (11.7%), that had an adverse effect were in the same study group of 20 exposed, at an extended duration of exposure (3 hr @ 1.0 ppm).

The responses of the human subjects, across the wide range of exposure levels (0.3 - 5.6 ppm) reported, indicates that individual groups, "sensitive" (asthmatic, emphysema, bronchitis, etc.), and non-sensitive receptors behave similarly and there is little or no basis for considering all asthmatics as a sensitive group. The true distribution of a truly

sensitive responder to NO<sub>2</sub> exposures is unknown and remains yet to be identified. However, a particularly exceptional effect or response frequently attributable to the sensitive groups is not demonstrated in the available human data reviewed. We could not identify a difference in response between the asthmatic and normal populations. The various human subjects tested, are represented at the low end as well as the high end of the exposure (concentration and duration) levels tested.

Figures 7-2 to 7-5 in the appendix are graphs of the probability of an adverse response occurring with increasing dose for a specified duration of exposure. They describe a probability of an effect occurring at some exposure level for 20, 30, 60 and 180 min. The categorical logistic regression analysis provides an estimate of the probability of some severity of response greater than a NOAEL according to the log concentration. From the log concentration the probability of an adverse effect response can be identified. The human toxicity database and the results of the statistical analyses are attached in the Appendix.

An examination of the raw and statistical analysis of the data indicates an onset of effects can occur among some at about 0.6 ppm during a one hour exposure. Reported clinical data and human responses from published citations and regulatory agencies suggest that 35-40 ppm may be an upper bound of NO<sub>2</sub> exposure. Within this concentration realm, there is the increased probability that a more serious adverse effect could occur together with a low probability of a potentially fatal effect.

## **7.9 DISCUSSION OF DATA**

Of the 557 human test subjects, four hundred and fifty (452) receptors (82%) failed to demonstrate any adverse effects from this range of exposures. They were put in the NOAEL group. One hundred and two (102) test subjects or 18% were observed to have had an adverse effect from exposure and were placed in the AEL group.

The results of categorical logistic regression analysis of the animal and human databases are in contrast to each other since one measures a severe effect, mortality while the human studies record the onset of effects. The animal mortality data is generated from very high levels of exposure, while the human data is developed from

low levels of NO<sub>2</sub> exposures. Although the very high concentration to experimental animals would expect to have greater impact, the duration of exposure was also shown to contribute to the impact of an adverse effect. Although the animal data was analyzed as a single entity, it indicated that the effects and the relationship between dose or duration to severity of response was significant. Haber's Law appears to be valid, even at higher levels, and is found to be consistent with the empirical data; duration and concentration contributed to the frank effect levels.

In the human studies, the impact of the duration and exposure concentration were included in the analysis. The graphs of human data exposure indicate that the 0.6-1.8 ppm levels of exposure appear to be important for onset of effects. And as expected, as duration is increased with dose, a higher probability of an adverse effect occurs. During a one hr exposure duration, the onset of an effect may be as low as 0.6 ppm whereas there is an increased probability of an AEL in some at the 1.6-1.8 ppm level of exposure. the number of adverse responses is slightly higher at the 4.0-5.6 ppm level of exposure. Thereafter, the curve plateaus showing a gradual lessening of the probability of any increase in the severity of effect with increasing duration. This is indicated by the 3 hours (180 minutes) curve. However, when analyzing the logistic regression analysis, the slope of the regression analysis is inconsistent with Haber's Law for the onset of human response data. This is probably due to the limited information base available for the exposures among humans.

## 7.10 CONCLUSIONS

**HCI:** The Air RISC Center of the USEPA Office of Air Quality, Planning and Standards prepared a health effects, dose response and regression analysis of short term exposure for HCI in 1991. This study was cited earlier in this report and supports the short term logistic regression model (STLRM) as a highly useful approach for estimating risks from short term. The review of additional citations and references, (Guth, 1996; Guth and Jarabek et al., 1991; Jarabek and Segal 1994) further supported the use of a logistic model for analysis of short term exposure risk assessments. The STLRM, provided adequate data is available and preferably using human data, has a significant potential for predicting the probability of response for some exposure dose of short term duration.

A recommendation for an ERF curve is included in Appendix A based on the Air RISC analysis.

**NO<sub>2</sub>:** In the case of the NO<sub>2</sub> data exposures, levels of 0.3-1.8 PPM appears to signal the onset of an observable adverse effect in humans. This is as would be expected, different people will respond differently due to inherently different susceptibilities. This susceptibility is not restricted to a particular group of receptors, but is spread across all human test subjects.

The logistic regression model analysis of the onset data was distinctly affected by observations at the one hour duration and led to rejection of Haber's Law even at these low doses of human exposures. The data appear to behave more consistently to a ceiling value for the on-set of a sensory response. The data, although informative, is limited and has such a wide spread that statistical analysis is not useful. Use of the derived logistic regression model for low dose response onset is thus not recommended when the database under analysis is restricted.

Analysis of the onset data did show, that there is no difference between the normal population of humans tested and those who have typically been suspected to represent a more sensitive population. There were 238 members of the human test subjects examined in the studies who were described as "asthmatics" (A) and had some respiratory illness. When their response to the NO<sub>2</sub> exposure was examined, it was found that 39 subjects (16%) demonstrated an observed adverse effect from these exposures. It suggests that these supposed sensitives, "the asthmatics etc.", do not respond similarly as a group but that there are degrees of response from among all test subjects, independent of the "asthma" or any other qualifying consideration.

Thus developing a specific ERF for a "sensitive population" is not recommended and not supported by the data examined. Data sufficient to expand the classes to more severe categories of response, other than the on-set of a statistically significant biological effects class, were not located. This is a pressing information gap and additional laboratory research in this subject area is highly recommended.

Expert opinion based on the limited observed effects in animal models can be useful in estimating the response profile of these more severe effects in humans but needs to be prepared very carefully. Logistic regression model analysis of the animal mortality studies revealed an equation that was consistent with observed data and corresponded with Haber's Law. Accordingly, this model has greater value for setting the frank effect level (mortality) ERF for NO<sub>2</sub>. Analysis of human onset of response effects at one hour exposures can be used to develop an ERF for mild effects. An ERF for a shorter (15 minute) duration would require a better understanding of the human response at these levels since the identified data is inconsistent. Although the data for NO<sub>2</sub> was not as consistent as that described by Air RISC for HCl, the analyses described above were combined to yield the NO<sub>2</sub> ERF proposed in Appendix A. This ERF appears reasonable in light of information previously developed, but must be used with care since it contains no arbitrary safety factors. Thus the curve presented is based upon both the analyzed data and professional judgment.

At any rate, the regression analysis of probability of some severity level of adverse effects from differing exposure levels at a fixed time appears to be a valuable tool for use in acute exposure risk analysis from rocket fuel combustion emissions. The database also indicates that extensive research data is needed to make the logistic regression model more effective as a tool when considering the exposures of concern to the Air Force. Fortunately, the database on NO<sub>2</sub> was sufficient to afford analysis and did provide information of whether it can be used in this manner.

**HNO<sub>3</sub>:** The database for HNO<sub>3</sub> is insufficient for analysis and requires additional experimental data at low and moderate levels of concentration for a fixed duration of exposure in an experimental animal species that is sensitive to inhaled toxicants, (e.g. guinea pig). Any gaps in the database or questions that remain for acceptable levels of exposure can be refined with experimental exposures among human volunteers.

**STLRM:** The short term linear regression model analysis of data indicates that at a given or fixed period of time, the levels of exposure can provide a picture of the probability of

some severity level of toxicity. Although useful in its present state, added human and/or animal data is required to provide a clearer, definitive picture of the probability of an increased level of severity of an adverse effects associated with increased exposure concentrations or duration. The human population response to brief exposures (15 minutes), needs to be developed further and any effects occurring at low levels of exposure for brief exposures clarified. These data will enhance the potential for use of the STLRM in estimating risks.

This report and the efforts by the EPA staff demonstrates the usefulness of the logistic regression model for short term exposure responses. However, it may require additional revisions and/or mathematical modifications. Also, an acceptable method of classification, designating severity levels from complex or multiple target organ toxicological data, will be especially necessary. It should be a consensus opinion from among the expert biomedical and scientific community. None the less, it will be an highly effective risk estimation tool, given that a usable database of information on which to conduct the analysis is available.

**FURTHER CONSIDERATIONS:** Another important point to consider is that, the Clean Air Act Amendments (CAAA) will apply to the Department of Defense (DOD) and their individual Services as well as to general industry. The DOD's use of this model to support their position will not by itself necessarily mean acceptance by the US EPA to satisfy the CAAA. Information developed using this model and an adequate amount of exposure data, would most likely be viewed in a positive light as an acceptable and supportive piece of evidence to defend the Air Force's position for estimating public health risks from such exposures.

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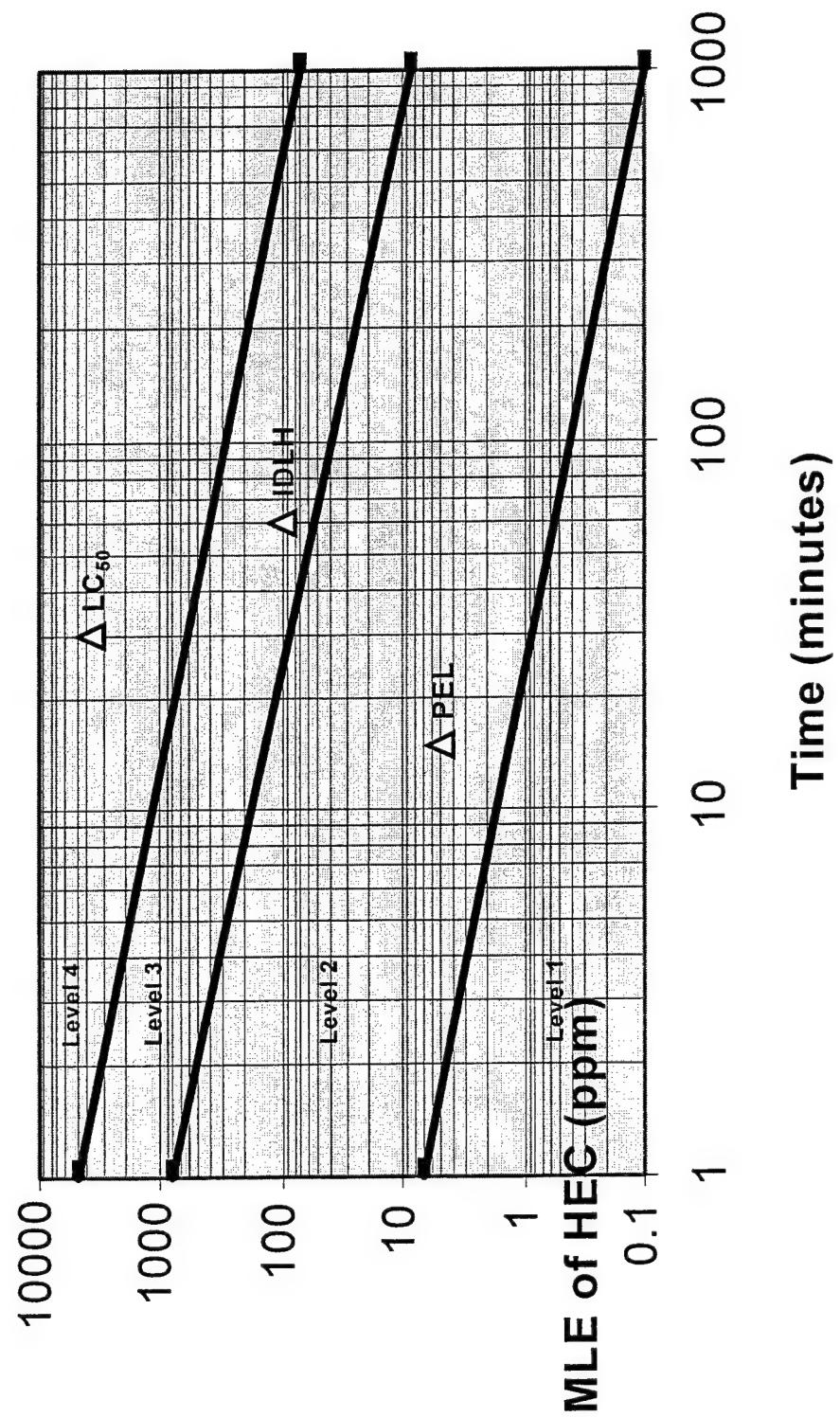
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## **APPENDIX A**

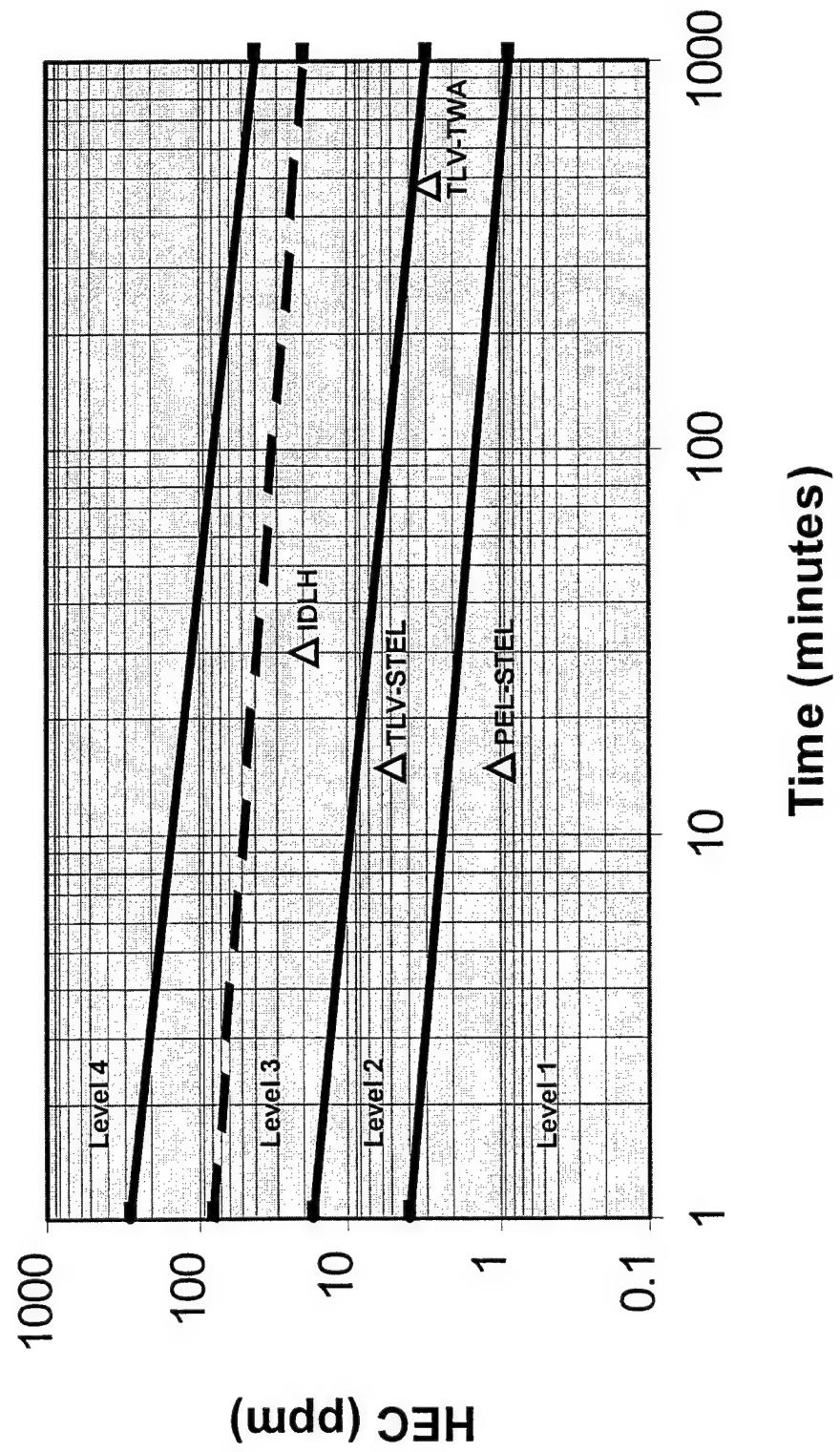
### **TOXICITY INFORMATION**

**Fig. 1.1 HCl Response Levels**

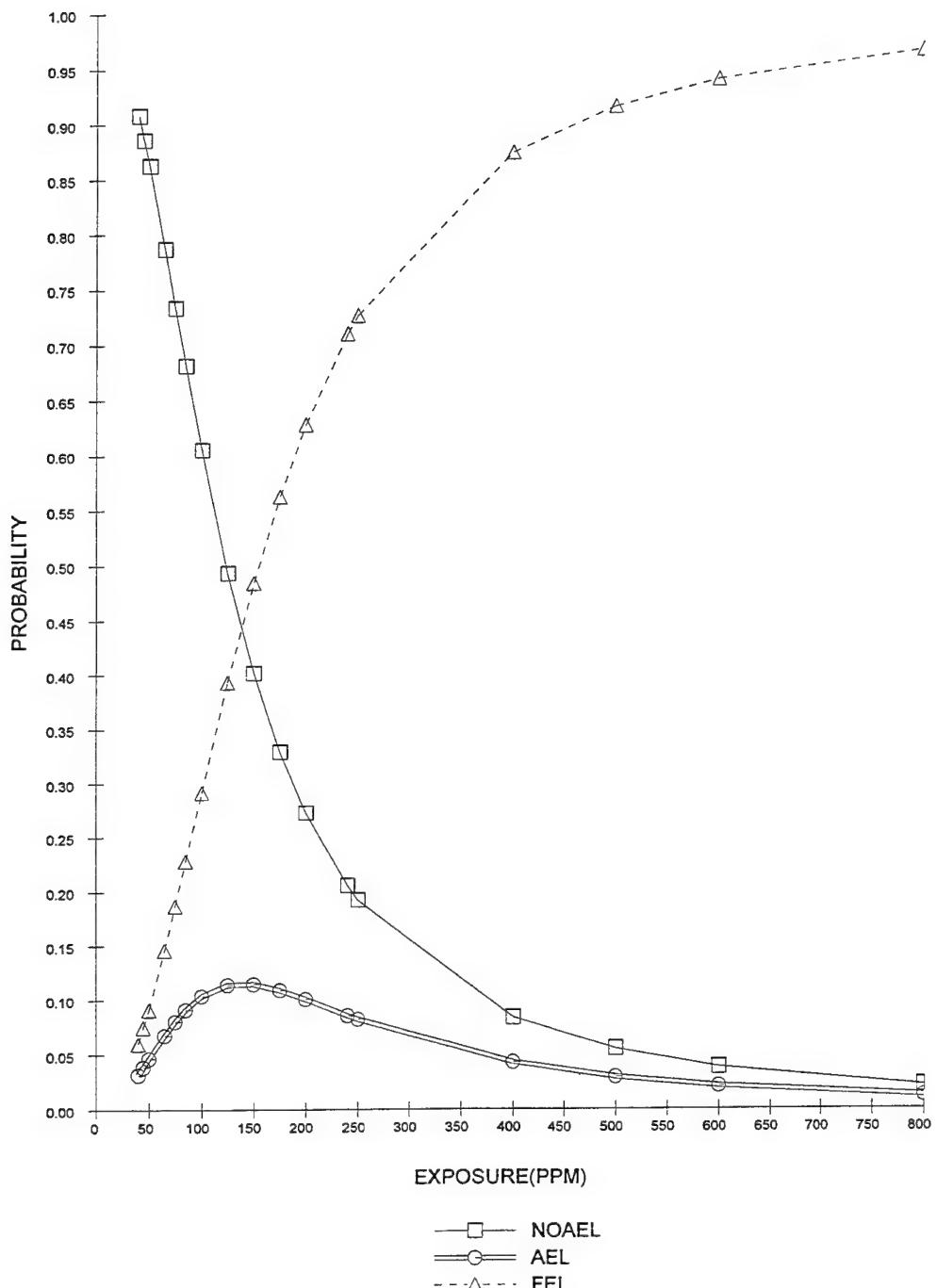


From: EPA 450/3-92-003, Jan. 1992

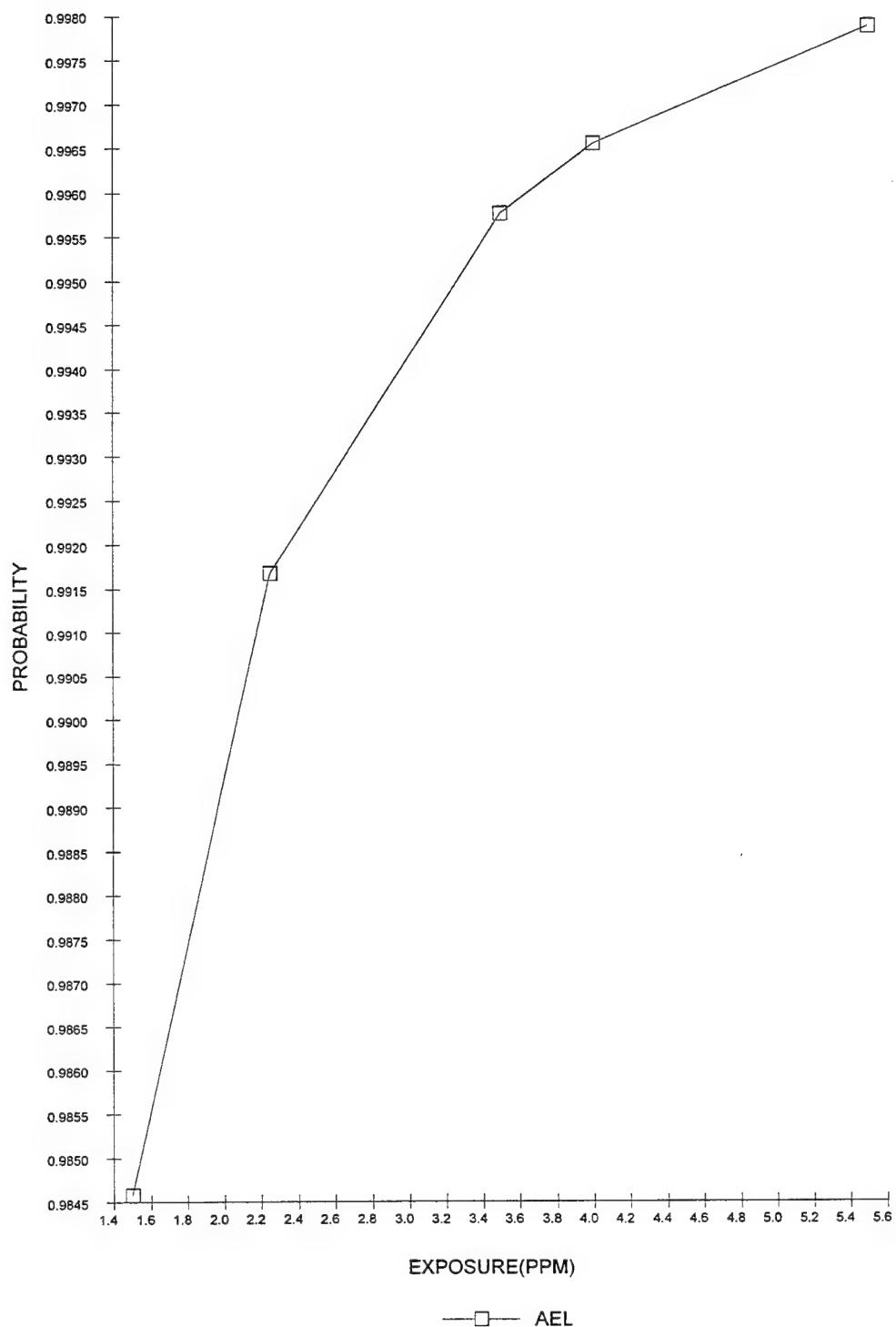
**Fig. 1.2 NO<sub>2</sub> Response Levels**



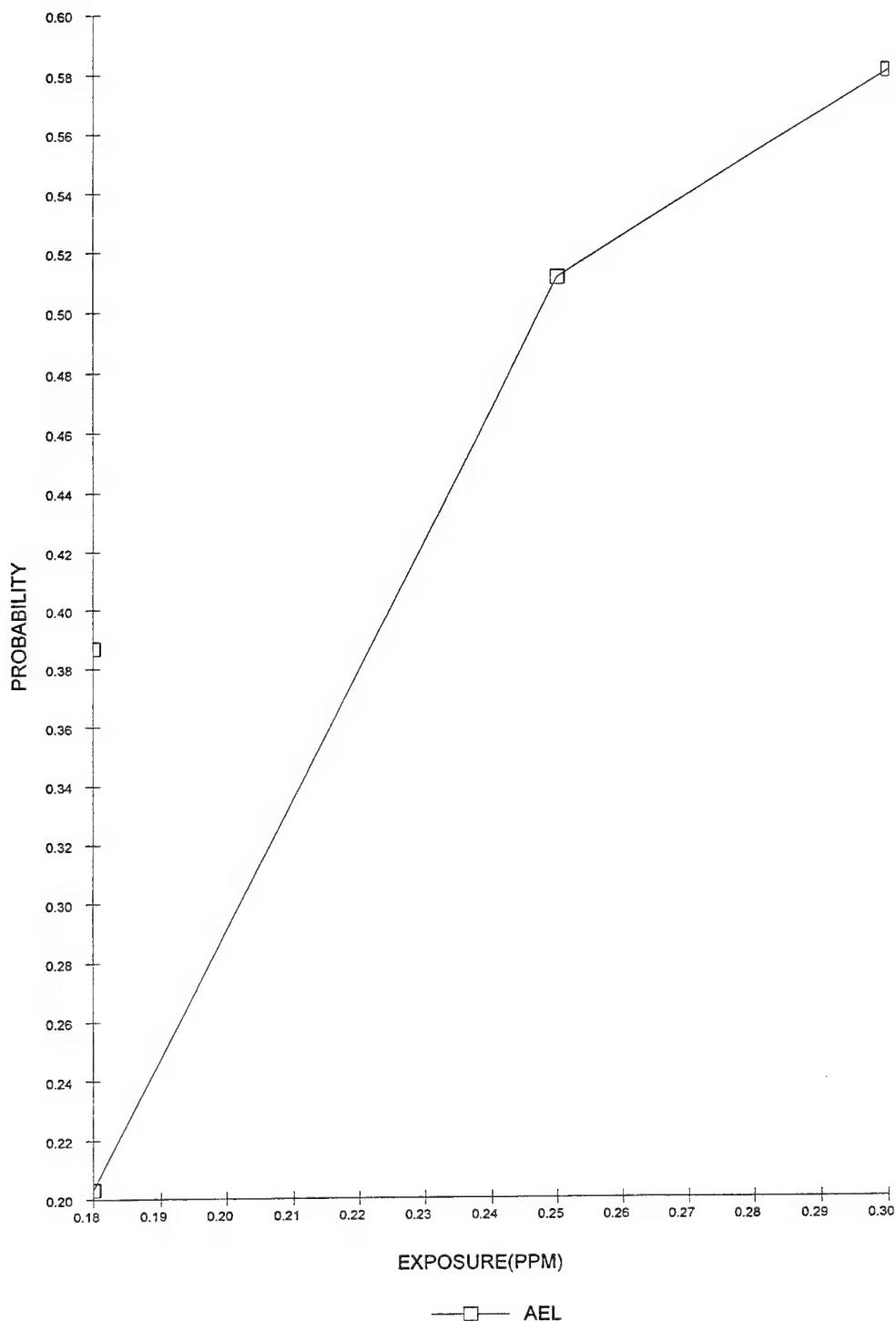
### ONE HOUR EXPOSURE



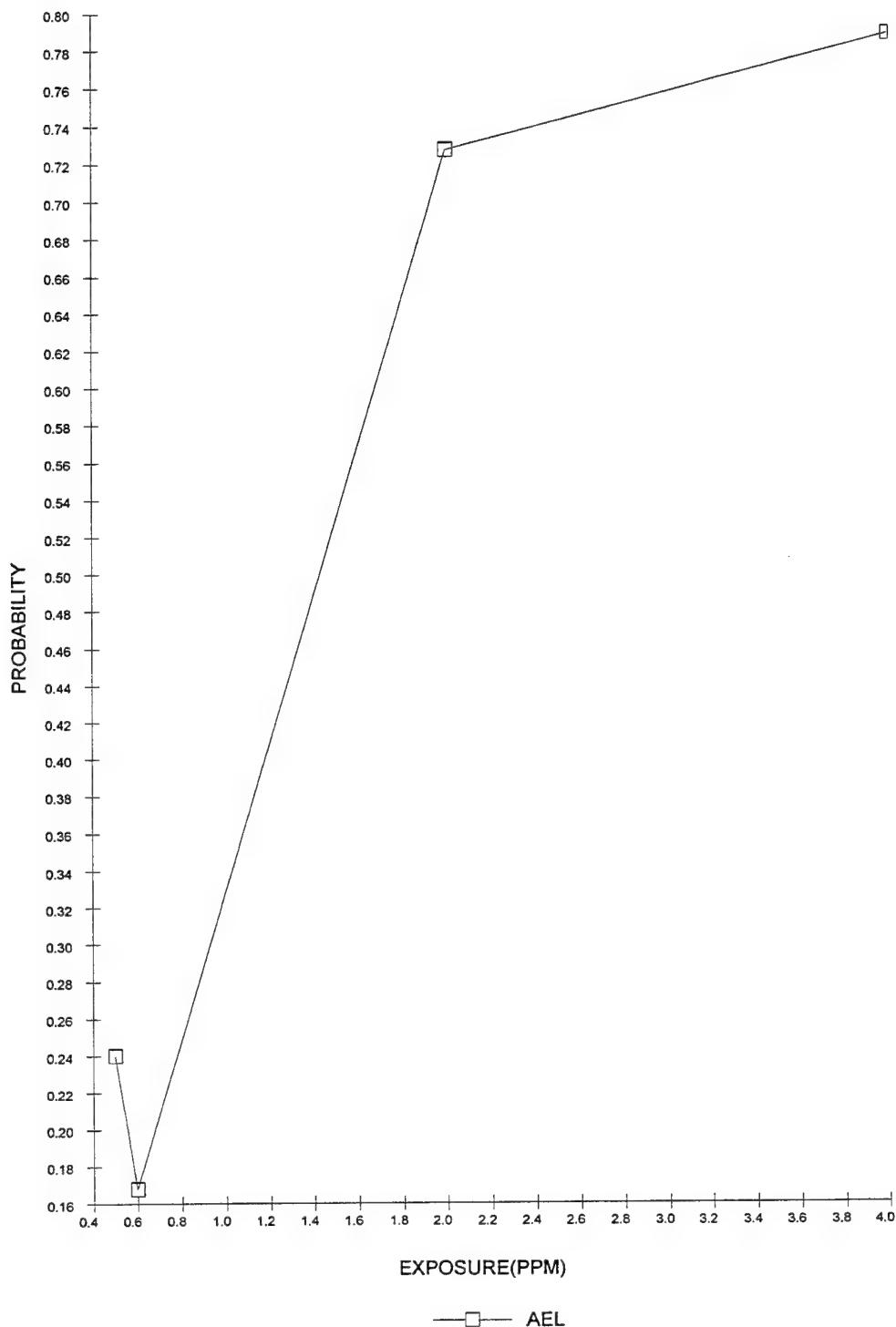
**PROBABILITY OF ADVERSE EFFECT FOR HUMAN EXPOSURE TO NO<sub>2</sub> FOR TWENTY  
MINUTES**



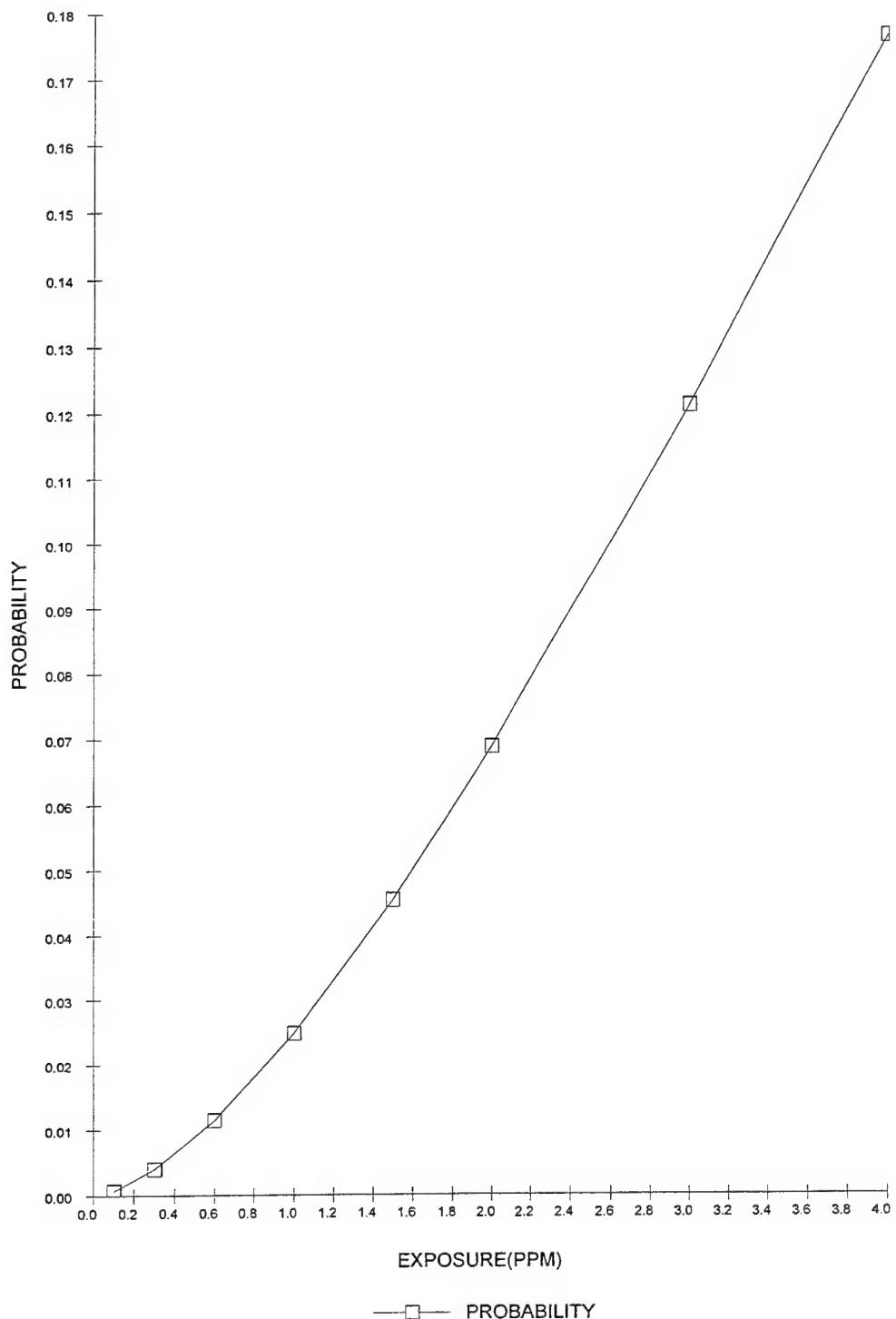
**PROBABILITY OF THE SEVERITY OF AN ADVERSE EFFECT AT INCREASING  
EXPOSURES FOR HUMAN EXPOSED TO NO<sub>2</sub> FOR ONE HALF HOUR**



**PROBABILITY OF THE SEVERITY OF AN ADVERSE EFFECT AT INCREASING  
EXPOSURES FOR HUMAN EXPOSED TO NO<sub>2</sub> FOR 1 HOUR**



PROBABILITY OF THE SEVERITY OF AN ADVERSE EFFECT AT INCREASING  
EXPOSURES FOR HUMAN EXPOSED TO NO<sub>2</sub> FOR THREE HOURS



## STATISTICAL ANALYSIS OF RAW ANIMAL TOXICITY DATA

Step 0-----

LOG LIKELIHOOD = -404.334  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 419.502 D.F. = 64 P-VALUE= 0.000

STANDARD COEFFICIENT	95% CI OF EXP(COEF)
ERROR	COEF/SE EXP(COEF) LOW-END HIGH-END

OUTCOME: NOAEL  
3 CONST1 -0.3764 0.952E-01 -3.95 0.69 0.57 0.83

NOAEL TO AEL  
4 CONST2 -0.7461 0.100 -7.45 0.47 0.39 0.58

### CORRELATION MATRIX OF COEFFICIENTS

CONST1	CONST2
3	4

CONST1	3	1.000
CONST2	4	0.831
		1.000

### COVARIANCE MATRIX OF COEFFICIENTS

CONST1	CONST2
3	4

CONST1	3	0.00907
CONST2	4	0.00793
		0.01003

STATISTICS TO ENTER OR REMOVE TERMS MODELS LOG LIKELIHOOD -404.3336

TERM	APPROX.		APPROX.		LOG LIKELIHOOD
	CHI-SQ. ENTER	D.F.	CHI-SQ. REMOVE	D.F.	
LEXP	101.39	1	0.0000	-353.6379	
LDUR	21.17	1	0.0000	-393.7468	

CONSTANT IS IN MAY NOT BE REMOVED.

ENTER TERM TO MOVE NEXT:

!V to View Output; ENTER to accept: LEXP --->  
STEP NUMBER 1 LEXP IS ENTERED

LOG LIKELIHOOD = -353.638

IMPROVEMENT CHI-SQUARE (2\*(LN(MLR)) = 101.391 D.F.= 1 P-VALUE= 0.000  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 318.111 D.F.= 63 P-VALUE= 0.000

	STANDARD COEFFICIENT	COEFFICIENT ERROR	95% CI OF EXP(COEF) COEF/SE EXP(COEF)	LOW-END	HIGH-END
ALL OUTCOMES					
1 LEXP	1.592	0.180	8.86	4.9	3.5
					7.0
OUTCOME: NOAEL					
3 CONST1	-7.915	0.862	-9.18	0.37E-03	0.67E-04
					0.20E-02
NOAEL TO AEL					
4 CONST2	-8.370	0.873	-9.59	0.23E-03	0.42E-04
					0.13E-02

#### CORRELATION MATRIX OF COEFFICIENTS

	LEXP	CONST1	CONST2
	1	3	4
LEXP	1	1.000	
CONST1	3	-0.992	1.000
CONST2	4	-0.992	0.997
			1.000

#### COVARIANCE MATRIX OF COEFFICIENTS

	LEXP	CONST1	CONST2
	1	3	4
LEXP	1	0.03228	
CONST1	3	-0.15376	0.74360
CONST2	4	-0.15554	0.75010
			0.76150

STATISTICS TO ENTER OR REMOVE TERMS MODELS LOG LIKELIHOOD -353.6379

TERM	ENTER	APPROX. CHI-SQ.	APPROX. D.F.	LOG P-VALUE	LIKELIHOOD
	REMOVE				
LEXP		101.39	1	0.0000	-404.3336

LDUR 8.19 1 0.0042 -349.5453  
CONSTANT IS IN MAY NOT BE REMOVED.  
ENTER TERM TO MOVE NEXT:  
!V to View Output; ENTER to accept: LDUR --->  
STEP NUMBER 2 LDUR IS ENTERED

---

LOG LIKELIHOOD = -349.545  
IMPROVEMENT CHI-SQUARE (2\*(LN(MLR)) = 8.185 D.F.= 1 P-VALUE= 0.004  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 309.926 D.F.= 62 P-VALUE= 0.000

STANDARD 95% CI OF EXP(COEF)  
COEFFICIENT ERROR COEF/SE EXP(COEF) LOW-END HIGH-END

ALL OUTCOMES

1 LEXP	2.037	0.248	8.20	7.7	4.7	12.
2 LDUR	0.3696	0.131	2.82	1.4	1.1	1.9

OUTCOME: NOAEL

3 CONST1	-9.808	1.14	-8.63	0.55E-04	0.59E-05	0.51E-03
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NOAEL TO AEL

4 CONST2	-10.27	1.15	-8.95	0.34E-04	0.36E-05	0.33E-03
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CORRELATION MATRIX OF COEFFICIENTS

---

LEXP	LDUR	CONST1	CONST2
1	2	3	4

LEXP	1	1.000			
LDUR	2	0.662	1.000		
CONST1	3	-0.994	-0.620	1.000	
CONST2	4	-0.994	-0.618	0.998	1.000

COVARIANCE MATRIX OF COEFFICIENTS

---

LEXP	LDUR	CONST1	CONST2
1	2	3	4

LEXP	1	0.06164			
LDUR	2	0.02154	0.01719		
CONST1	3	-0.28064	-0.09249	1.29228	
CONST2	4	-0.28333	-0.09308	1.30262	1.31813

STATISTICS TO ENTER OR REMOVE TERMS      MODELS LOG LIKELIHOOD -349.5453

TERM	ENTER	APPROX. CHI-SQ.	APPROX. D.F.	CHI-SQ. ENTER	D.F.	P-VALUE	LOG LIKELIHOOD
LEXP		88.40	1	0.0000		-393.7468	
LDUR		8.19	1	0.0042		-353.6379	
CONSTANT				IS IN		MAY NOT BE REMOVED.	
ENTER TERM TO MOVE NEXT:							
!V to View Output; ENTER to accept: NONE      --->							

NO TERM PASSES THE REMOVE AND ENTER LIMITS ( 0.1500 0.1000 ).

#### SUMMARY OF STEPWISE RESULTS

STEP	TERM	LOG NO. ENTERED	IMPROVEMENT REMOVED	GOODNESS OF FIT DF	LOG LIKELIHOOD	CHI-SQUARE	P-VAL	CHI-SQUARE	P-VAL
0		-404.334		419.502	0.000				
1	LEXP		1	-353.638	101.391	0.000	318.111	0.000	
2	LDUR		1	-349.545	8.185	0.004	309.926	0.000	

TOXICOLOGICAL DATABASE OF ANIMALS EXPOSED TO NO<sub>2</sub>

SPECIES	EXP	DUR	NOAEL	AEL	FEL
GP-M/F	40	1	6	0	0
MUS-SW-M	40	1	13	0	0
Rat-LE-M	40	1	6	0	0
MUS-SW-M	45	3	10	0	0
Dog-M/F	50	1	1	0	0
GP-M/F	50	1	6	0	1
MUS-SW-M	50	1	5	0	0
Rabbit-M/F	50	1	4	0	0
Rat-LE-M	50	1	17	0	0
Dog-M/F	50	2	2	0	0
GP-M/F	50	2	5	0	1
MUS-SW-M	50	2	5	0	0
Rat-LE-M	50	2	12	0	0
Rat-LE-M	65	2	9	0	0
Wistar rat/F	75	0.17	3	6	0
Dog-M/F	75	1	2	0	0
GP-M/F	75	1	3	0	1
MUS-SW-M	75	1	5	0	1
Rabbit-M/F	75	1	7	0	1
Rat-LE-M	75	1	28	0	3
Dog-M/F	75	2	2	0	0
GP-M/F	75	2	1	0	3
MUS-SW-M	75	2	4	0	2
Rabbit-M/F	75	2	6	0	0
Rat-LE-M	75	2	11	0	1
Rat-LE-M	85	1	6	0	6
Dog-M/F	100	0.5	2	0	0
GP-M/F	100	0.5	1	0	1
MUS-SW-M	100	0.5	8	0	2
Rabbit-M/F	100	0.5	2	0	1
Rat-LE-M	100	0.5	5	0	0
GP-MF-M	100	1	0	0	2
MUS-SW-M	100	1	2	0	8
Rabbit-M/F	100	1	4	0	0
Rat-LE-M	100	1	2	0	3
Dog-M/F	100	2	2	0	1
Rat-LE-M	100	2	0	0	8
MUS-SW-M	125	0.083	6	0	0
Wistar rat/F	125	0.17	2	6	1
Rabbits	125	0.17	3	0	0
MUS-SW-M	125	0.5	2	0	4
MUS-SW-M	125	1	0	0	6
Rabbit-M/F	150	0.083	2	0	0
GP-MF-M	150	0.5	1	0	3

Rat-LE-M	150	0.5	8	0	2
Dog-M/F	150	1	0	0	2
Rabbit-M/F	150	1	5	0	1
Rat-LE-M	150	1	3	0	10
GP-MF-M	150	2	0	0	3
Rabbit-M/F	150	2	2	0	2
Rat-LE-M	150	2	2	0	10
Wistar rat/F	175	0.17	0	8	1
Rabbits	175	0.17	2	1	0
MUS-SW-M	200	0.083	2	0	4
Rabbit-M/F	200	0.083	2	0	0
Rat-LE-M	200	0.083	6	0	6
MUS-SW-M	200	0.17	0	0	6
Rabbit-M/F	200	0.17	1	0	1
Rat-LE-M	200	0.17	4	0	8
Dog-M/F	200	0.33	0	0	2
MUS-SW-M	200	0.33	0	0	6
Rabbit-M/F	200	0.33	2	0	2
Rat-LE-M	200	0.33	0	0	5
GP-MF-M	200	1	0	0	2
Rat-LE-M	240	0.33	0	0	4
Rat-LE-M	250	0.083	2	0	2
Rat-LE-M	250	0.17	2	0	2
Rabbits	250	0.17	0	6	0
Rat-LE-M	250	0.33	0	0	4
Rabbits	400	0.17	0	3	0
Sheep-F-lung only	500	0.29	0	5	0
Sheep-F-nose only	500	0.29	5	0	0
Rabbits	600	0.17	0	3	0
Rabbits	800	0.17	0	1	2

STATISTICAL ANALYSIS OF HUMAN TOXICITY DATA FOR NO2 EXPOSURE (BMDP statistical package)

STEP NUMBER 0

LOG LIKELIHOOD = -265.186  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 425.111 D.F.= 27 P-VALUE= 0.000

STANDARD 95% CI OF EXP(COEF)  
COEFFICIENT ERROR COEF/SE EXP(COEF) LOW-END HIGH-END

OUTCOME: NOAEL

3 CONST1 1.495 0.110 13.6 4.5 3.6 5.5

STATISTICS TO ENTER OR REMOVE TERMS MODELS LOG LIKELIHOOD -265.1863

TERM	APPROX. CHI-SQ. ENTER	APPROX. D.F. REMOVE	LOG D.F. P-VALUE	LOG LIKELIHOOD
LEXP	48.71	1	0.0000	-240.8316
LDUR	89.93	1	0.0000	-220.2208
CONSTANT		241.79	1 0.0000	-386.0830
CONSTANT		IS IN		MAY NOT BE REMOVED.

STEP NUMBER 1 LDUR IS ENTERED

LOG LIKELIHOOD = -220.221  
IMPROVEMENT CHI-SQUARE (2\*(LN(MLR))) = 89.931 D.F.= 1 P-VALUE= 0.000  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 335.180 D.F.= 26 P-VALUE= 0.000

STANDARD 95% CI OF EXP(COEF)  
COEFFICIENT ERROR COEF/SE EXP(COEF) LOW-END HIGH-END

OUTCOME: NOAEL

2 LDUR 1.437 0.163 8.80 4.2 3.1 5.8  
3 CONST1 1.230 0.121 10.2 3.4 2.7 4.3

CORRELATION MATRIX OF COEFFICIENTS

LDUR CONST1  
2 3

LDUR 2 1.000  
CONST1 3 0.011 1.000

## COVARIANCE MATRIX OF COEFFICIENTS

LDUR CONST1  
2 3

LDUR 2 0.02668  
 CONST1 3 0.00021 0.01464

STATISTICS TO ENTER OR REMOVE TERMS      MODELS LOG LIKELIHOOD -220.2208

TERM	APPROX.		APPROX.		LOG LIKELIHOOD
	CHI-SQ.	D.F.	CHI-SQ.	D.F.	
	ENTER	REMOVE	P-VALUE		
LEXP	37.42	1	0.0000	-201.5112	
LDUR		89.93	1	0.0000	-265.1863
CONSTANT		115.35	1	0.0000	-277.8954
CONSTANT		IS IN		MAY NOT BE REMOVED.	
STEP NUMBER	2	LEXP		IS ENTERED	

LOG LIKELIHOOD = -201.511  
IMPROVEMENT CHI-SQUARE (2\*(LN(MLR)) = 37.419 D.F.= 1 P-VALUE= 0.000  
GOODNESS OF FIT CHI-SQ (2\*O\*LN(O/E)) = 297.761 D.F.= 25 P-VALUE= 0.000

STANDARD COEFFICIENT	95% CI OF EXP(COEF)				
COEFFICIENT	ERROR	COEF/SE	EXP(COEF)	LOW-END	HIGH-END

#### OUTCOME: NOAEL.

OUTCOME: NOCL	1 LEXP	-0.6684	0.113	-5.92	0.51	0.41	0.64
2 LDUR		1.421	0.178	8.00	4.1	2.9	5.9
3 CONST1		1.086	0.128	8.47	3.0	2.3	3.8

### CORRELATION MATRIX OF COEFFICIENTS

LEXP LDUR CONST1  
1 2 3

LEXP	1	1.000		
LDUR	2	-0.088	1.000	
CONST1	3	0.061	-0.093	1.000

COVARIANCE MATRIX OF COEFFICIENTS

---

	LEXP	LDUR	CONST1
	1	2	3

LEXP	1	0.01277	
LDUR	2	-0.00176	0.03155
CONST1	3	0.00088	-0.00213
			0.01646

STATISTICS TO ENTER OR REMOVE TERMS      MODELS LOG LIKELIHOOD -201.5112

---

TERM	APPROX.		APPROX.		LOG LIKELIHOOD
	ENTER	CHI-SQ.	D.F.	CHI-SQ.	
ENTER	REMOVE	P-VALUE			
LEXP		37.42	1	0.0000	-220.2208
LDUR		78.64	1	0.0000	-240.8316
CONSTANT		75.25	1	0.0000	-239.1350
CONSTANT	IS IN			MAY NOT BE REMOVED.	

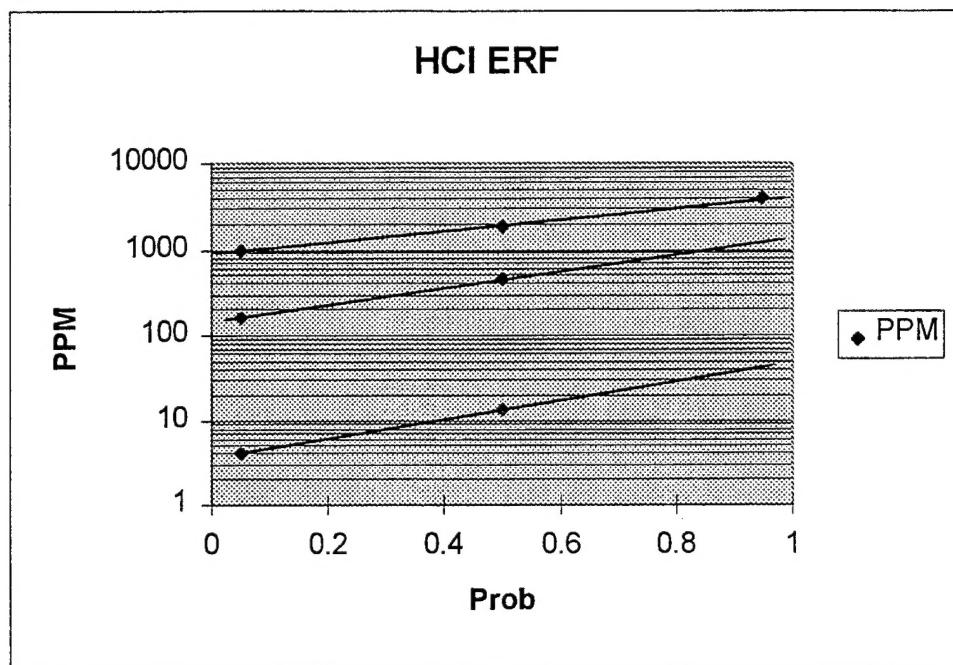
SUMMARY OF STEPWISE RESULTS

STEP	TERM	LOG	IMPROVEMENT	GOODNESS OF FIT
NO.	ENTERED	REMOVED	DF	LIKELIHOOD CHI-SQUARE P-VAL CHI-SQUARE P-VAL
0		-265.186		425.111 0.000
1	LDUR	1	-220.221	89.931 0.000 335.180 0.000
2	LEXP	1	-201.511	37.419 0.000 297.761 0.000

TOXICOLOGICAL DATABASE OF HUMANS EXPOSED TO NO<sub>2</sub>

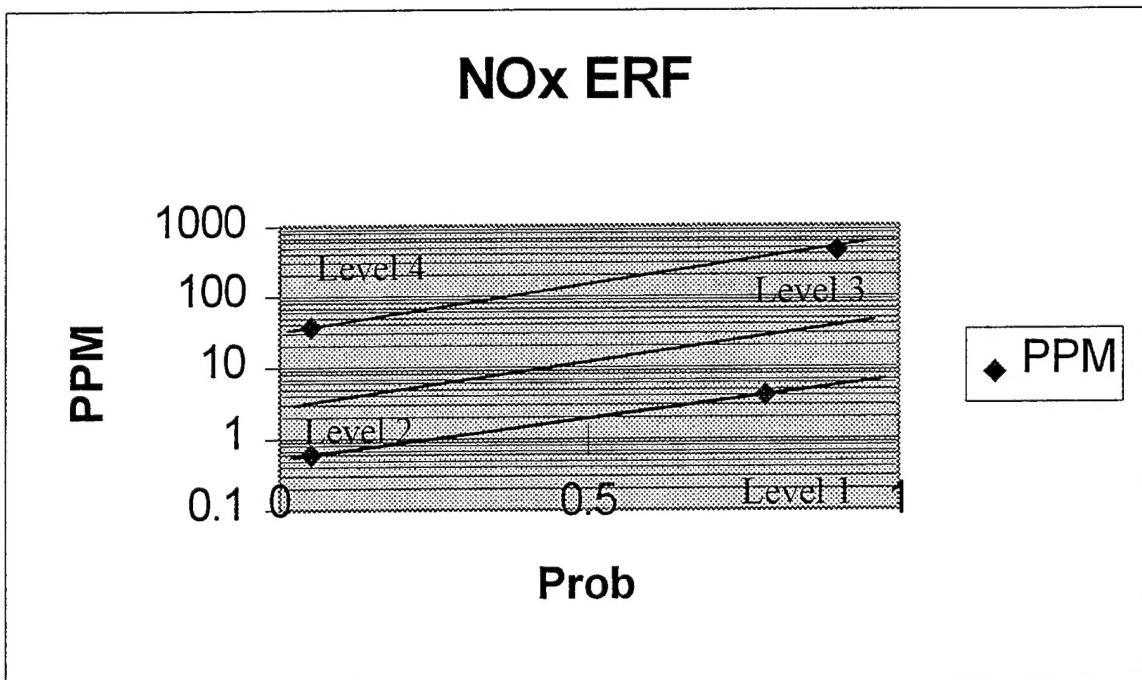
SPECIES	EXP	DURATIO	No.	Subject	NOAE			AE	FEL	GROUP	SE
					N	s	L				
Human-	1		3		20		12	8	0		0
N/A											
Human	0.1		3		20		20	0	0		0
Human	0.6		2		8		8	0	0		0
Human	0.3		2		59		59	0	0		0
Human	0.6		2		59		59	0	0		0
Human-A	0.3		3		34		34	0	0		0
Human-A	0.5		1		10		0	10	0		1
Human-A	0.5		1		10		10	0	0		0
Human	2		1		18		18	0	0		0
Human-	0.18		0.66		20		20	0	0		0
N/A											
Human-A	0.2		2		31		31	0	0		0
Human-	4		1.25		48		48	0	0		0
N/A											
Human-	0.6		8		5		5	0	0		0
N/A											
Human	3		3		6		6	0	0		0
Human	4		3		4		4	0	0		0
Human-F	0.6		2		21		21	0	0		0
Human-M	1		2		5		5	0	0		0
Human-A	0.25		0.5		11		11	0	0		0
Human	0.18		0.5		9		9	0	0		0
Human	0.3		0.5		9		9	0	0		0
Human-A	0.6		1.25		21		21	0	0		0
Human-A	0.3		0.5		13		7	6	0		0
Human-A	0.3		0.5		15		0	15	0		1
Human	0.6		3		9		9	0	0		0
Human	1.5		3		15		0	15	0		1
Human	2		3		15		15	0	0		0
Human-	2.3		5		14		14	0	0		0
M/F											
Human	2.25		0.33		8		0	8	0		1
Human	4		0.33		8		0	8	0		1
Human	5.5		0.33		8		0	8	0		1
Human	1.5		0.33		8		0	8	0		1
Human	3.5		0.33		8		0	8	0		1
Human	3.5		4		8		0	8	0		1

Candidate Exposure Response Function  
One Hour Exposure - Hydrogen Chloride



- One hour exposure developed from EPA 450/3-92-003, table 9, fig. 2 and fig. 3; 5% probability of an effect on total lung surface selected
- Assumes logistics curve remains valid at higher exposure levels
- Moderate response levels developed from animal data converted to human equilivant concentrations, original authors determined the threshold to moderate classification
- Time weighted averages are thought to be valid only within a single category of response
- Curves contain no uncertainty factor for conversion from animal to human response and the 5% probability accepts a relatively high level of likely injury; no other safety factors are included

Candidate Exposure Response Function  
NO<sub>x</sub> - One Hour Exposure



Conditions:

- Population includes normal individuals and those with chronic lung disease, elderly and smokers; it may not represent the hyperresponsive which have not been evaluated with NO<sub>x</sub> exposures.
- Level 1 is the no adverse effect level determined from statistical analysis of one hour human exposure studies.
- Level 2 responses are based on empirical observations of onset of statistically significant biological effects; they are considered to be minor responses but represent an awareness of exposure.
- Level 3 response area was set by professional judgment. Empirical data is lacking on the transition from minor to moderate responses. Toxicological observations in animals were assessed which represent transition to a higher probability of adverse biological effects to establish the level and the slope was selected to match the fatal effects curve. Empirical validation of this curve is recommended.
- Level 4 fatal effects area was determined from statistical analysis of multiple species animal data; it was not adjusted for human equivalent concentrations.
- Level 2 responses are considered to be sensory response types and probably are reversible
- Time weighted averaging within a category of response should be appropriate but not between categories of response
- A 5% probability of response was selected as the threshold for each response level, there are no safety factors included in the curves